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EDITORIAL

HEALTH OF LIVESTOCK AND THE WORLD'S FOOD SUPPLY

We draw our readers' attention to a report of a meeting recently held in London at the request of the Food and Agriculture Organisation, at which veterinarians from several countries discussed animal health problems and their control, especially those epizootic in character. The important recommendation from the meeting is that F.A.O. should set up a Standing Veterinary Committee to advise the Organisation. It is of the greatest importance at this time in the history of the world, when plans are being discussed for means of ensuring the availability of food for peoples of all nations, that the supply of animal products receives appropriate consideration; and, further, that the health of livestock is considered so that the maximum supplies of such products can be forthcoming. Throughout the world the toll of animal life and the non-productivity of livestock from disease, much of which may be preventable by the adoption of modern methods of control, must be enormous. It can readily be understood that, in some parts of the world, the application of control measures present difficulties: ways and means, however, will have to be found to overcome them. Such work would seem, logically, to come into the scope of the activities of F.A.O., and we welcome the recommendation that a Standing Veterinary Committee be set up.

While we in this country recognise the limiting factors from diseases of livestock in milk and meat production, and realise how the livestock industry is benefiting now and will, we hope, benefit to greater extents from the carrying out of control measures, we may have but a poor conception of the results of the ravages of the epizootics which are prevalent in different parts of the world and which sweep huge areas with disastrous results. By various means, many of these conditions can be controlled: now is the time to make the necessary arrangements to put the machinery into motion. By collaborative effort on the part of the various nations concerned, much can be done to check the losses and to ensure a healthier livestock throughout the world. When such devastating diseases have been controlled the time will be ripe to undertake the many other means to improve

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quality and, therefore, productivity of animal products. Not only do such epizootics interfere greatly with the quantity of available animal products; they also, in some countries, have considerable bearing on the products of the land, for animals, such as cattle and buffaloes, play a big part in the cultivation of the soil, and, therefore, their numbers and health in these countries are all-important.

We also draw attention to the results of the combined work of scientific workers from America and Canada on the new method of controlling rinderpest, an account of which has recently been published; summaries of the various papers are reproduced in this issue of the VETERINARY JOURNAL. This work is of first-rate importance and stands as an example of what can be done when a determined effort is made to study a problem. The combination of workers well versed in techniques, from different countries, has resulted in the production of a weapon against rinderpest which might go very far in controlling, and even eliminating, that disease from all countries in which it is found. There seems little doubt that the future of disease investigation and the evolving of methods of control lies in the carrying out of research work under similar conditions as those pertaining on the island in the St. Lawrence River. Not only are workers familiar with the disease required, but also scientifically trained personnel with knowledge which can be applied to the problem must form part of the team of investigation. What has been done for rinderpest can be done for other diseases: with the adoption of such methods of research, results are bound to follow—results, the application of which may well solve some of the world's greatest livestock problems.

We note from the Press announcement of the meeting referred to above that part of the recommendation of the Committee is that the Standing Veterinary Committee should co-operate with the International Office in Paris and should consider the possibility of correlating the existing veterinary bodies under central international machinery in Paris. If this can be brought about a great achievement will have been accomplished and time will be gained, for much ground has already been covered. There is need for such correlation in order that the best results may be obtained and that information can be pooled. Various organisations, especially the International Office, have already begun the correlation envisaged: this can be extended and their activities can be strengthened. It will be for the suggested Standing Veterinary Committee to work out the ways and means of linking up these bodies in such a way that the best results will be obtained.

We look forward with keen interest to the result of the deliberations of the F.A.O. Conference, recently held in Copenhagen, at which the above recommendations were considered. If they have been adopted, veterinarians with the able assistance of workers in other branches of science have a huge task upon which to embark. The task, however difficult, is capable of being carried out, and if it is tackled in the proper spirit will, in the years to come, lead to results of untold benefit throughout the whole world.

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GENERAL ARTICLES

PULLORUM DISEASE: Diagnosis and Immunisation

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Literature

SINCE the isolation of *Salmonella pullorum* by Rettger and Stoneburn in 1909 from the ova and ovaries of adult hens and from the yolk sac of chicken, many workers have tried to investigate the problem of pullorum infection, susceptibility and control of the disease.

Doyle (1928) found about 3 per cent. of eggs laid by infected hens contained *S. pullorum.*

Jones (1912) showed that transmission of infection occurred in three ways: (1) through the egg, (2) by direct contact with infected chicks, and (3) by placing chicks in contaminated premises. He proved that *S. pullorum* is passed in the droppings of infected chickens and that the susceptibility of young birds is reduced when they are two to three weeks of age.

Mathews (1928), however, failed to find *S. pullorum* in mature infected hens. He believes that hens may become infected through feeding upon infected eggs.

Wernery and Lerche (1931) state that chicks are infected in the egg and that infection is spread among hatched chicks. Attempts to infect adult hens with *S. pullorum* culture gave positive results, and cases of natural infection among hens were reported, though experiments carried out by Brunett (1930) on this point gave negative results.

Brunett (1931) reported that he failed to spread infection from affected to healthy hens in the absence of the cock. In another experiment, however, the same author found that the male is unnecessary for the spread of infection.

Proscholdt (1934) proved by controlled experiments that an infected cock may play an important rôle in spreading the disease to healthy fowls. Evidence of infection was based upon the agglutination test and upon culture from cloacal material. Pheasants were found susceptible to natural infection during the first week when their eggs were incubated in infected incubators (Hendrickson and Hilbert, 1931).

Brunett (1931) found that eight turkeys out of a flock of 157 gave positive reactions to the agglutination test with *S. pullorum* antigen. *S. pullorum* was isolated from the ovary of a turkey hen which gave an agglutination titre of 1 : 320. The results of this experiment demonstrate the possibility that infected adult turkeys may propagate infection on a farm after the disease has been eradicated from fowls. According to this author, in any scheme for the eradication of pullorum disease it would appear advisable to include turkeys.

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Villani (1937) tested the susceptibility of various domestic and wild birds to artificial infection with *S. pullorum*. Pheasants, finches, sparrows, turkeys, geese, ducks and doves were readily infected, death following in 24 hours. In every case it was possible to recover the organism from the heart blood. The lesions varied, but there was always an intense enteritis and diarrhœa. Doves appeared to be the most susceptible. It was concluded that wild birds may suffer from a natural infection and may act as carriers of the disease.

According to Gaiger and Davies (1938), rabbits and guinea-pigs can be fatally infected and kittens made severely ill by ingestion.

Olney and Bederke (1928) found that rabbits died from *S. pullorum* infection after feeding them with infected eggs incubated for 18 days.

Wagner (1934) failed to infect pigs by feeding them with carcasses of hens which had died from *S. pullorum* or by feeding them with cultures of *S. pullorum;* even subcutaneous inoculation of suspensions failed to produce the disease.

It is now agreed that the disease causes high mortalities in young chicks in the first two weeks, and that the disease occurs in adult hens causing no or very low mortality. Infection occurs by the passage of the organisms through the egg, by infected incubators or by the contamination of the premises in poultry farms by droppings of infected chicks and carriers.

S. pullorum affects young chicks acutely and produces a chronic pathological condition in adults. Mortality in chicks is very high, sometimes amounting to 80 per cent., while adults rarely die from the disease. They act as carriers, disseminating the micro-organisms in the eggs.

The symptoms in young chicks are drooped wings, ruffled feathers and most constant and diagnostic diarrhœa, which is white or yellowish in colour. The fæces dry around the cloaca, forming plugs of chalk-like material which may close the opening. The diarrhœa is sometimes fœtid. Depression and extreme weakness result and the bird dies within two to three days.

Stafseth and Johnson (1927) reported atypical symptoms in an outbreak of white diarrhœa in chicks. The birds developed abnormal thirst to the extent that they remained at the water fountains continuously. This was followed by a watery, brownish diarrhœa. The chicks invariably swelled up and became very large. The affected birds died after four to six days of the illness. Death sometimes occurred so rapidly that the naked-eye lesions were absent.

In sub-acute cases, however, there is catarrhal enteritis with thickening of the intestinal wall; the cæca contain a semi-solid yellowish mass; the liver is sometimes enlarged and the lung often shows yellowish nodules of varying sizes. The yolk is not absorbed and may be full-sized.

In adult fowls, in addition to enteritis, small necrotic foci may be found in the liver, spleen, pancreas and the heart, in acute cases. In chronic cases the lesions are confined to the ovaries, the ova are abnormal in appearance and size, being angular, flattened and reddish-green in colour; degenerated and normal ova may be seen alongside. Litzke (1934) found 20 per cent. of fowls suffering from inflammation of the oviduct and degeneration of the ovarian follicles to be infected with *S. pullorum*. In an outbreak among mature fowls, Hart and Hungerford (1936) found that the most outstanding lesions were enlargement of the liver with

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miliary small necrotic areas. Pronounced pericarditis and myocarditis with miliary abscesses in the myocardium were constant in all cases. They succeeded to isolate *S. pullorum* from the examined birds. Edwards and Floyd (1927) found that one of the most common abnormalities in affected hens was the presence of cysts in the ovaries, these cysts resulted from the closing of the stigmal rupture of the calex. They gave them the name of follicular cysts or corpus luteum cysts.

Stafseth and Johnson (1927) reported the post-mortem findings in affected chicks, having watery œdematous fluid, sometimes gelatinous, in the abdominal and thoracic cavities as well as in the subcutaneous tissue. The heart was very thin-walled and flabby, the liver light yellow and very friable. There was usually unabsorbed yolk of a liquid or cheesy consistence.

Isolation of the organism is one of the diagnostic and conclusive tests of pullorum disease. As the organism is found in the fæces together with many other coliform bacilli, gram-positive and gram-negative bacilli, it may be difficult to isolate the organism in a pure culture, without special technique. Most of the work done in this connection was to determine the best dye and its accurate concentration which is sufficient to inhibit contaminants and does not affect *S. pullorum*. Ito (1934) tested the action of 18 sulphur compounds on the growth of *S. pullorum*. He found that sodium thiosulphate and sodium sulphate were the best to favour the growth of the organism. Thus, he advises a medium composed of 2 per cent. agar to which 0.5 per cent. of one of the above-mentioned compounds as the best suitable media for *S. pullorum*.

Mallmann, Thorp and Semmes (1928) found that brilliant green had a selective inhibitory effect upon E. coli and other contaminants normally present in the fæces. He thus recommends brilliant green liver infusion agar for the isolation of *S. pullorum* and allied organisms from the tissues or fæces of dead chicken.

Gaiger and Davies (1938) agree with the previous authors in the value of brilliant green in the inhibition of coliforms and other contaminants which are usually found in fæces. They advise 1 : 50,000 concentration in ordinary agar as the most suitable media for the isolation of *S. pullorum* and *Shigella gallinarum*.

Kernkamp (1929) advises that cultures should be made from the liver, heart blood or yolk of the dead chicken, as he found it easier and more successful to isolate the organism from those organs than from fæces where it is mixed with many other contaminants which may interfere with or overcome *S. pullorum* in culture.

For the diagnosis of pullorum disease in adult carriers the agglutination test is employed, and although authors differ in the titre which should be considered as diagnostic, they all regard it as a most reliable test. The efforts of different investigators is to find a simpler and more accurate test to replace the agglutination test, since it is not always easy to take a sufficient quantity of blood from a fowl for the test.

, Miessner and Schutt (1931) reported a rapid whole blood agglutination test which can be made on the farm. It consists of mixing one drop of blood with a drop of *S. pullorum* suspension on a slide and reading the results after a few seconds. They pointed out, however, that the test is open to various faults unless done by special technique and the results read by an expert.

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Edgington and Broerman (1931) dealt with the comparison of the agglutination test and the intradermal pullorin test. They found that the agglutination test is much more reliable and that a positive reaction of a titre of 1 : 25 should be considered indicating infection. From a bacteriological survey they came to the conclusion that the pullorin test was not so efficient as the agglutination test.

Fleischhaner (1931) came to the conclusion that the slow agglutination test is by far the test of choice for the detection of infection and that the rapid whole blood method, although simple and can be carried out on the premises, has not really anything to be recommended. Dilution of 1 : 50 is considered indicating the infection. Lahaye (1931) pointed out that the pullorin test is unreliable and of little practical value. The tube agglutination test, although slow and laborious, has proved to be a test of great accuracy.

Bunyea and Hall (1932) carried out a comparative test between the standard tube agglutination method and the stained antigen rapid whole blood test. The tests agreed in 91 per cent., but the agglutination method was found more accurate. Leynen and Willems (1932) do not agree with these authors, and state that they used the rapid plate agglutination test for two years and found it more accurate than the tube method.

Warrack and Dalling (1931), however, found that the tube agglutination test was more reliable than the pullorin test and that the stained antigen test with whole blood detected fewer infected birds than the ordinary agglutination test.

Barger and Torrey (1933) claimed that the rapid whole blood agglutination test is almost as reliable as the tube test for the detection of fowls infected with *S. pullorum* and that it has the advantage over the tube method of being quicker and cheaper.

Biely and Roach (1932) compared the whole blood, the rapid serum and the tube agglutination tests and found that the three tests agreed in about 94 per cent. of cases. Thus, they state that the three tests are equally efficient for diagnostic purposes.

Rievel (1934) found that the rapid whole blood test and the tube agglutination test were in agreement in 90 per cent. of cases.

Biely and Lloyd (1934) stated that the rapid whole blood test may be as accurate as the tube agglutination test if it is used by an experienced and well-trained veterinarian.

Van Roekel and Clarke (1935) concluded from a series of experiments that the whole blood agglutination test is not as efficient as the tube agglutination test. If efforts are made to increase its efficiency it would be very helpful and desirable.

Stafseth (1938) used the whole blood slide agglutination with a stained concentrated antigen.

Winter (1939) found the tube test and the whole blood plate test in agreement in 99 per cent. of cases when the titre was as high as 1 : 50 dilution; when infection was mild there was only 87 per cent. agreement. Thus the tube agglutination test appeared to be more reliable in detecting mild infection.

Newsom, Floyd and Alford (1925) state that a positive reaction to the agglutination test may be considered a highly accurate indication of infection, whereas a negative reaction does not indicate freedom from pullorum infection, either recently acquired or long standing.

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Olney and Bederke (1928) made post-mortem examinations on birds which had previously given positive and negative reactions to the tube agglutination test. They found that the test is very reliable and useful in controlling the disease.

Bushnell and Brandly (1929) drew attention to the fact that the addition of too much phenol to the antigen used in the tube agglutination test causes a precipitate which makes the test impossible to read correctly, and that too little preservative allows certain phenol-fast organisms to grow in the tubes—thus leading to false interpretations. They consider a complete agglutination at a titre of 1 : 50 as positive. Birds showing a lower titre were considered as negative reactors. These authors state that the rapid slide agglutination test is as effective as the tube test and may be used to replace it.

Konno and Zaizen (1933) tried the complement fixation and precipitation tests for the diagnosis of *S. pullorum* infection and found them to agree in a large proportion of the cases.

Kester (1931) claims that for practical purposes the pullorin test is superior to the agglutination test for the diagnosis of *S. pullorum* in fowls. If the test is carried out properly and good pullorin is used it is as accurate as the agglutination test; the birds are handled less, reactors are less likely to be overlooked and less equipment is necessary. This opinion, however, does not agree with that of most workers on the subject.

Edgington and Broerman (1931) carried out experiments to compare the agglutination and pullorin tests. They came to the conclusion that the intradermal pullorin test is not so efficient as the agglutination test.

Lahaye (1931) does not agree with the previous authors, and states that in his experience the pullorin test is more reliable than the tube agglutination test. This is contrary to the experience of Leynen and Willems (1932), who found that the tube agglutination test is by far more accurate than the pullorin test. Warrack and Dalling (1931) agree with the previous author in considering the tube agglutination test to be superior to both pullorin test and the rapid whole blood agglutination test.

Bunyea (1934) compared the rapid whole blood agglutination, pullorin and the slow tube agglutination tests. Post-mortem and bacteriological examinations were performed on the birds tested. Agreement between the rapid whole blood test and the bacteriological findings was more favourable than that between the pullorin test and the bacteriological findings. The results with the slow test supported the reliability of the whole blood test. From these results, it was concluded that the pullorin test is not a satisfactory method for the diagnosis of pullorum infection.

Edwards and Floyd (1927) summarised the work of different authors on the comparison between pullorin and agglutination tests. They carried out experiments on different types of pullorins in conjunction with the tube agglutination test. The tests were controlled by post-mortem examination and the cultures from the ovaries of the examined hens. From the results of these tests it appeared that the agglutination test was a much more accurate index of infection than the pullorin test.

Bushnell and Brandly (1929) studied the correlation between different pullorins and tube agglutination tests. They used a commercial pullorin composed

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of a dried product (probably an alcoholic precipitate of old cultures of the organism grown in broth) suspended in salt solution before use and another type composed of bacterial suspension in salt solution. Positive reactions consisted of a persistent swelling about the size of that originally made by injecting the pullorin. From these experiments it was concluded that the pullorin test in its present state is not so satisfactory in detecting carriers of bacillary white diarrhœa as is the agglutination test. Since there was found some correlation between the two reactions, it is evident that there is a possibility of developing a pullorin which will be satisfactory for the purpose. Until such a product is developed, the agglutination test should be used.

All authors working on the subject agree that the best way to control pullorum infection is to remove reactors and to provide good hygiene. Incubators should be properly disinfected and the young chicks kept on raised wire-mesh floors out of reach of excreta, etc. The eggs of reactors should not be used for incubation. It is advisable to test the flock annually and remove new reactors.

Gage and Flint (1928) state that the elimination of bacillary white diarrhœa from a breeding flock depends on two factors: (1) The detection of infected birds through the application of blood serum agglutination test; and (2) the removal of these birds from the flock and the protection of the growing flock from infection. One without the other is practically useless.

Experimental

The aim of the present work is to investigate the methods that have been used for the diagnosis of pullorum disease and to modify them in such ways as to render them more easily carried out and more applicable in the field, and at the same time to be as reliable as possible.

It was also intended to attempt immunisation of the mature birds by vaccination so that immunity might be passed through the egg to the hatching chick.

I. *Diagnosis of Pullorum Infection by the Agglutination Test.*

Ten mature chicken were artificially infected with *S. pullorum*, each receiving 0.1 c.c. of a 24-hours broth culture, inoculated intramuscularly. Ten other normal chicken were used as controls.

The tube agglutination test was performed on the serum of the infected chicken. These invariably agglutinated pullorum suspension to a very high titre. The control chicken were all negative.

These two batches of birds were employed in this work.

The rapid (slide) whole blood agglutination test: The rapid slide agglutination test was first tried. One drop of whole blood from each of the infected and normal chicken separately was mixed with a drop of pullorum suspension; after thorough mixing, the slides were left for 15 minutes, after which the results were read. No definite clumping or flocculation could be clearly seen, even by the aid of a lens. When this experiment was repeated using a stained antigen (brilliant green or gentian violet 1 : 5,000) the same unsatisfactory results were again encountered. The plasma and the red cells of the infected birds became granular in appearance very much the same as occurred in the non-infected controls.

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Thus, the rapid whole blood agglutination test as carried out by this method could not be relied upon for diagnosis.

So far the most reliable method for diagnosis of this disease by agglutination is the standard slow serum tube method. The following modification, however, has been tried and gave quite accurate and satisfactory results.

The tube whole blood agglutination test: Here a drop or two of whole blood are added to measured quantities of a fairly thick citrated pullorum suspension in narrow test tubes (0.85 per cent. saline containing 0.4 per cent. sodium citrate). The tubes were incubated at 37 deg. C. overnight before the results were read.

When reading the results we depend mainly on the degree of clarity or otherwise of the supernatent fluid and not on the clumped deposit, since the blood cells will naturally mask the agglutinated bacteria. Obviously the titre obtained by this method must be correlated with that obtained if serum only was used, as in the case of the standard method of agglutination.

For this purpose the following series of experiments were made:—

(1) *Determination of the agglutinating titre of citrated plasma of the infected birds.*

Two c.c. of blood were drawn from a wing vein, to which sodium citrate was added to make 0.4 per cent. final concentration. After shaking well, the tubes were left to allow the blood cells to settle down. The plasma was then pipetted off and used for agglutination in the usual way. The average highest titre obtained was 1 : 640.

(2) *Determination of the agglutinating titre of serum of the same infected birds.*

When agglutination was repeated on the same birds but using serum, the average highest titre was 1 : 2,560.

Thus the serum titre is four times that of plasma.

(3) *Determination of the volume of blood cells and its ratio to plasma in birds.*

Since we are going to use whole blood containing both plasma and cells in the test proper, it was necessary to determine the volume of the blood cells and its ratio to the plasma.

For this purpose 2 c.c. of blood were drawn from a wing vein of each of five birds and added to 2 c.c. citrated saline (final concentration 0.4 per cent.) in a narrow, graduated cylinder and allowed to settle. It was found that the average volume of the deposited cells was 1 c.c. *Thus, the blood of birds contains half its volume cells and the other half plasma.*

Therefore, when carrying out the agglutination test with citrated whole blood, the final titre must be *multiplied by two to get the corresponding titre of plasma.* Then this must be divided by four to get the corresponding titre of serum.

Thus, the final agglutination titre of whole blood must be divided by two to get the corresponding final serum titre.

(4) *The test proper.*

(a) *Pullorum antigen.—S. pullorum* was seeded on agar slopes and incubated at 37 deg. C. for 48 hours. The growth was washed off to make a heavy, even suspension in citrated saline (0.85 per cent. sodium chloride and 0.4 per cent. sodium citrate) containing 0.5 per cent. carbolic acid. The suspension was shaken repeatedly at intervals for two days, the grosser particles allowed to fall by setting

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aside for half an hour and the supernatent fluid pipetted off and standardised to the proper density by comparison with Brown's standard opacity tube No. 4. The suspension was then kept in the refrigerator.

(b) *Standard loop.*—A platinum wire was made into a loop, the outer diameter of which was 7 mm. One c.c. contains 36 loopfuls of this standard loop.

(c) *Technique.*—Measured quantities of antigen were placed into a series of long, narrow test tubes (Wassermann tubes will do well) as follows: 1, 1.5, 2 and 3 c.c. The wing vein of the bird to be tested is pressed to raise the vein, when it is pricked with a sharp needle. A loopful of blood was taken, added to each tube and thoroughly shaken. A further tube containing suspension only was used as control. The tubes were then incubated at 37 deg. C. overnight before the results were read. When the test was first tried control positive (artificially infected) and control negative birds were used.

Complete clarity of the supernatent fluid occurred in all the tubes of the infected birds, indicating complete agglutination, while in the negative control birds all the tubes remained as turbid as the control tubes, containing suspension only.

In determining the titre of agglutination, we calculate as follows:—

	○ 1 c.c.	○ 1.5	○ 2	○ 3	○ Control 2
Suspension	36 loopfuls	54	72	108	—
Blood	1 loopful	1	1	1	—
Dilution of whole blood	1/36	1/54	1/72	1/108	—
Dilution of plasma ...	1/72	1/108	1/144	1/216	—
Dilution of serum ...	1/18	1/27	1/36	1/54	—

From the diagram, it will be seen that when one loopful of blood is added to varying amounts of citrated suspension, dilutions of whole blood will be 1/36, 1/54, 1/72 and 1/108 respectively. Since it was shown by experiment that the blood of birds contains half its volume cells, then the corresponding dilutions of plasma will be 1/72, 7/108, 1/144 and 1/216 respectively. And since it was proved that the agglutinating titre of serum is four times that of plasma, then the corresponding serum dilutions will be 1/18, 1/27, 1/36 and 1/54 respectively.

When the agglutination test was done on the serum of the same birds by the usual slow serum tube method the same agglutinating titre were obtained, thus showing the accuracy of this modified method.

Since it is agreed that a positive serum titre of 1/25, with partial agglutination of 1/50 indicates the infection, therefore it would be only feasible to use the dilutions in the second and fourth tubes, which correspond to serum dilutions of 1/27 and 1/54, to save antigen, time and the blood of birds.

This method has been used in testing 200 birds in a poultry farm (Faculty of Agriculture, Giza) and has given clearcut, easily read results. Complete agreement was obtained when the original agglutination tube method was performed for confirmation.

Table I shows the results of agglutination recorded when the two methods were performed together on the same birds:—

TABLE I.

Agglutinating serum titre of the same bird when serum only or whole blood was used.

No.	Variety and Sex	Tube serum agglutination						Whole blood aggl. in terms of serum	
		1/10	1/20	1/40	1/80	1/160	1/320	1/27	1/54
2973	Balady cock ...	+	+	±	—	—	—	+	±
3535	„ hen ...	+	+	+	+	+	+	+	+
2963	Fayoumy hen ...	+	+	+	+	±	—	+	+
973	Turkey „ ...	+	+	+	+	±	—	+	+
961	„ „ ...	+	+	+	±	—	—	+	+
1922	Pullet	+	+	+	±	—	—	+	+
3273	„	+	+	+	+	±	—	+	+
3330	„	+	+	+	+	+	±	+	+
4030	„	+	+	+	+	+	±	+	+
188	Fayoumy hen ...	+	+	+	+	+	±	+	+

+ = Agglutination.
± = Partial agglutination.
— = No agglutination.

In addition to the above kinds, samples of serum and whole blood were tested from the following, all with completely negative results: 17 Balady cocks, 28 Balady hens, 42 Fayoumy hens, 2 Turkey cocks, 3 Turkey hens, and 98 Pullets.

The advantages of the method described are as follows:—

(1) The technique is simple and the results obtained are clearcut and easily read, and can be carried out by any breeder.

(2) No use of pipettes or test tubes for dilution, which means less work in the laboratory. Dilutions here, however, are made by adding equal drops of blood to varying amounts of citrated antigen.

(3) Only two drops of blood are needed, thus avoiding taking bigger amounts of blood to clot and separate its serum.

(4) The citrated antigen may be measured in tubes and kept ready for use.

(5) Results are obtained in 24 hours.

(6) The method is most suitable for field work when a large number of birds are to be tested.

(7) This modified method of agglutination may be applied to the diagnosis of other diseases in other animals, e.g., brucellosis, after standardising the loop and the quantity of antigen in accordance with the accepted positive titre of the particular disease.

II. *Diagnosis of Pullorum by the Pullorin Test.*

Preparation of Pullorin.—S. pullorum was seeded on agar slopes and incubated at 37 deg. C. for 15 days. The growth was washed off with normal saline to make a fairly thick suspension. The suspension was then divided into

three equal quantities and each was separately treated so as to make three kinds of pullorin which are intended for testing on known positive and negative birds for the sake of comparison.

Pullorin type A.: 0.5 per cent. phenol was added to the suspension and left at 37 deg. C. for three days. Sterility tests were made before its use.

Pullorin type B : Here the suspension was killed by heat, one hour at 60 deg. C. in a water bath, and then 0.5 per cent. phenol was added as a preservative.

Pullorin type C : The bacterial suspension was treated with normal sodium hydroxide for one hour at 37 deg. C. to lyse the bacterial cells. The extract was then neutralised with normal hydrochloric acid, using phenol red as indicator. Then 0.5 per cent. phenol was added as a preservative.

The three kinds of pullorin were finally diluted with carbolised saline to the same density of about two million organisms per c.c.

Each of these pullorins was tested on five positive and also on five negative birds previously tested by the agglutination test.

0.1 c.c. pullorin was injected intradermally in the right wattle. For control, 0.1 c.c. carbolised saline was similarly injected in the left wattle. The birds were examined after 6, 12 and 24 hours after inoculation. *No reaction could be detected.* There was complete absence of swelling, heat or tenderness at the seat of inoculation, neither in the affected nor in the normal birds. Thus, the three kinds of pullorin failed to be of any diagnostic value in pullorum infection.

III. *Diagnosis of Pullorum by Isolating the Causative Organism.*

In chicks dying from pullorum, the most conclusive method of diagnosis is by isolating the causal organism at post-mortem examination and confirming its identity by fermentation tests and by agglutinating a known pullorum serum. Cultures are usually made from the heart blood, liver, yolk sac or bone-marrow. For such a purpose a selective medium would be of great value. Such media should allow the growth of *S. pullorum* and at the same time inhibit the growth of coliform bacteria which are the usual contaminants in the blood of chicks dying from pullorum.

The Effect of Various Dyes on the Growth of S. pullorum and E. coli.—In order to ascertain the most suitable dye for this purpose, the following experiment was made :—0.1 per cent. stock solutions of the following dyes were prepared : methylene blue, gentian violet, brilliant green, basic fuchsin.

Each dye was incorporated in ordinary agar in the following series of concentration : 1 : 5,000, 1 : 10,000, 1 : 20,000, 1 : 40,000, 1 : 80,000 and 1 : 160,000.

Agar slopes containing these concentrations were made. Two sets were prepared from each dye. One set was inoculated with a loopful of *S. pullorum* (a 24-hours' broth culture diluted 100 times). The other set was similarly seeded with *E. coli*. Ordinary agar slopes containing no dye were inoculated with each organism as control. All the slopes were then incubated at 37 deg. C. for 24 hours before the results were recorded.

Table II shows the results obtained, from which it will be seen that the best selective dye was gentian violet.

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TABLE II.

The Effect of Dyes on the Growth of Salmonella pullorum and E. coli.

Dye	Organism	Concentration of dyes 1/5,000	1/10,000	1/20,000	1/40,000	1/80,000	1/160,000	Control
Methylene blue	Pullorum	++	+++	+++	++++	++++	++++	++++
	Coli	++	+++	+++	++++	++++	++++	++++
Gentian violet	Pullorum	—	±	+	++	+++	+++	++++
	Coli	—	—	—	—	+	+++	++++
Brilliant green	Pullorum	—	+	++	+++	+++	+++	++++
	Coli	—	—	++	++	+++	+++	++++
Basic fuchsin	Pullorum	+	++	++	+++	++++	++++	++++
	Coli	+	++	++	+++	++++	++++	++++

N.B.—Results read 24 hours after inoculation, tubes being incubated at 37 deg. C.

++++ = luxuriant growth
+++ = moderate growth
++ = slight growth
+ = scanty growth
± = partial growth
— = no growth

For, while this dye in a concentration of 1 : 40,000 allowed a moderate growth of *S. pullorum*, it completely inhibited the growth of *E. coli*.

Methylene blue and the basic fuchsin failed as selective dyes since both organisms grew freely in their presence, even in a concentration as high as 1 : 5,000.

Brilliant green, which has been highly recommended by many workers in selective media, did not prove as selective as gentian violet. The former, however, in a concentration of 1 : 10,000, inhibited the growth of *E. coli* and allowed a scanty growth of *S. pullorum;* in concentrations of 1 : 20,000 and downwards free growth of both organisms occurred.

Therefore, the dye of choice to be recommended in selective media for the isolation of *S. pullorum* is gentian violet in a concentration of 1 : 40,000.

To confirm this finding, the experiment was repeated using agar plates containing the same concentration of dye. A plate was inoculated with *E. coli*, another with *S. pullorum*, and a third plate was inoculated with a mixture of the two organisms. Clear-cut results were obtained. *E. coli* was completely inhibited, while *S. pullorum* grew luxuriantly in the same concentration of dye.

The use of McConkey's medium for the isolation of Salmonella pullorum.—As *S. pullorum* is a non-lactose fermenter, it was thought of interest to attempt the use of McConkey's medium for its isolation. Since, in the majority of cases, *S. pullorum* is usually accompanied with coliform organisms, which are normal inhabitants of the intestinal tract and invade the blood after death, this medium might prove of value both as a selective and a differential one. For this purpose, three plates of McConkey's medium were used. One plate was seeded with *E. coli*,

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another with *S. pullorum*, and the third with a mixture of the two organisms. The plates were incubated at 37 deg. C. for 24 hours and the character of the growth was noted. On this medium *S. pullorum* grew well, although the colonies were much smaller and more translucent than those of *E. coli*. Most important, however, was the colour of the colonies of the two organisms: coli colonies were pink in colour, being lactose fermenters, while pullorum colonies were colourless. This medium may, therefore, be employed for the isolation of *S. pullorum*, the colonies of which are small, translucent and colourless as compared with coliform colonies, which are big, opaque and reddish in colour.

Gentian violet agar, however, is preferable for this purpose since it completely suppresses the coliform organisms and at the same time allows the free growth of pullorum.

IV. *Immunisation Against Pullorum Disease.*

Professor Morcos suggested this research with the view of producing immunity in adult chicken in order to protect them from contracting the disease without becoming carriers. In addition, such immune adult birds may transmit immunity through their eggs to the hatching chicks and thus be protected from incidental infection during the early stage of life.

With these objects in view, the following experiment was carried out:—

Twenty just mature chicken (two cocks and 18 hens) were tested by the agglutination test, and all gave negative reactions. They were inoculated with a heat-killed vaccine (containing a thousand million organisms per c.c.). Each bird received 0.5 c.c., 1 c.c. and 1 c.c. intramuscularly at weekly intervals.

At the first injection the birds were off food and dull for one day, after which they were quite normal. The second and third doses of vaccine did not cause any ill effects.

Two weeks after the last injection, the blood of the vaccinated birds as well as that of five non-vaccinated controls (a cock and four hens) was tested by the agglutination test. The vaccinated birds gave a very high positive titre ranging between 1 : 320 and 1 : 1,280. The controls, however, did not exceed a titre of 1 : 10.

The vaccinated and control birds were placed in separate cages, given serial numbers and kept under the best hygienic conditions until they laid eggs. The eggs were collected and given the number of their parents as well as the date of laying.

One hundred eggs laid by the vaccinated birds and 25 by the non-vaccinated controls were incubated in a clean and disinfected incubator. The hatched chicks of both batches of eggs were given the homologous number of the egg. Eighty chicks of the vaccinated batch and 19 of the non-vaccinated control survived. The dead chicks were post-mortemed and cultures were made from the heart blood, spleen and liver, from which no organisms were isolated.

When the chicks were two months old the blood of the vaccinated batch as well as that of the control was tested by the agglutination test. The vaccinated gave a titre between 1 : 80 and 1 : 320, while the non-vaccinated did not exceed

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1 : 5, thus showing that agglutinins were transmitted from the vaccinated mother to the chick through the egg.

Summary and Conclusions

(1) A modified method of the agglutination test for the diagnosis of pullorum disease has been adopted. A drop of blood is added to a measured quantity of citrated antigen in a narrow test tube and incubated overnight at 37 deg. C. The reading of the result is judged by the clarity or otherwise of the supernatent fluid. The technique is simple and the results obtained are clear-cut, easily read and have been found most reliable when confirmed by the standard serum tube method. This modified method is quite safe and may be strongly recommended for field work when a large number of birds are to be tested.

(2) The intradermal pullorin test failed to be of any diagnostic value in pullorum infection.

(3) A selective medium, consisting of agar containing a 1 in 40,000 solution of gentian violet has proved very efficient for the isolation of *S. pullorum*. In this medium the gentian violet inhibits the growth of lactose-fermenting organisms.

(4) McConkey's medium was tried for the isolation of *S. pullorum*. On this medium, the colonies of this organism are small, colourless and translucent.

(5) The results of protective inoculation against pullorum disease have given very promising results. The agglutinin titre rose very high in the vaccinated adult birds, denoting the acquirement of a good degree of immunity. It has also been shown that agglutinins are transmitted from the immune adult to the chick through the egg.

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POULTRY POST-MORTEM TECHNIQUES

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Introduction

BECAUSE of the comparatively low value of the unit in a poultry flock, and of the non-specific character of clinical symptoms displayed by ailing birds, post-mortem examinations are used to a very much greater extent than in almost any other kind of farm stock. It appears that during the next few years even more attention will be given to the subject. Every animal species presents special problems of clinical and laboratory diagnosis and it is essential to know the conditions most likely to be encountered and the simplest method of exact diagnosis. The subsequent account is based on experience gained when working at the Seale-Hayne Agricultural College, Newton Abbot, Devon, in the laboratory of Mr. C. V. Watkins, M.R.C.V.S., D.V.S.M., the Veterinary Investigation Officer for the South-Western Provinces.

Techniques Adopted

These are conveniently grouped under the following six headings:—

(1) Preparation, examination for external parasites, and opening of carcase.
(2) Examination of organs *in situ*.
(3) Examination for internal parasites.
(4) Bacteriological examination (primary).
(5) Completion of post-mortem examination.
(6) Specific laboratory techniques.

(1) *Preparation, Examination for External Parasites, etc.*

Note the condition of the bird, and whether it was killed for examination or allowed to die. Spreadeagle on its back on a suitable-sized wooden board—for adults a good working size is 18 in. by 13 in. to take a single bird, and for chicks 12 in. by 10 in. to take two or three birds; boards are slightly raised at one end. Secure the bird in position by means of nails through the wings and feet, or by tapes; for chicks, use wood- or bone-handled pins, which are durable and may be readily sterilised.

Whilst handling the bird at this stage the tibio-tarsal joint (commonly called the "hock") is examined for the condition known as *slipped hock*, in which the tendons are found obviously displaced to one side; where this condition occurs in a number of birds simultaneously in a flock it represents a mineral deficiency condition which is known as *perosis*.

Any excessive number of lice (*Menopon, Lipeurus* and *Goniocotes* spp.) or fleas (*Ceratophyllus gallinæ*) will be noticed as they tend to seek the warmth of the operator's hands. Examine for *red mites* (*Dermanyssus gallinæ*), smaller than lice and darker in colour; these are important parasites capable of causing death; they will often crawl or drop out onto the board and should be identified by microscopical examination. The feet and legs are examined for the crusts

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caused by scaly leg mites (*Sarcoptes mutans, v. gallinæ*); and the feet for swellings underneath, known as *bumble foot,* which are often caused in the first instance by some slight injury. Suspected scaly leg may be confirmed by soaking or heating the crusts in 10 per cent. caustic potash solution, teasing apart, and examining under the ⅔ objective.

The comb and wattles are examined for lesions of *favus,* a mould infection due to *Achorion gallinæ;* for the necrotic skin lesions of *fowl pox,* an important virus disease causing severe eruptions; and for the swelling sometimes seen in cases of fowl typhoid. The eye sockets are examined for swelling which occurs in the *oculo-nasal* form of *fowl pox;* also the eyes for the *pearly eye* condition associated with *Neurolymphomatosis,* although this is often difficult to detect after death. Examine the vent and note the colour of any diarrhœa which may be present; also a contagious purulent condition known as *vent gleet.* in which a very mixed bacterial flora is present.

Soak the feathers or down with 5 per cent. lysol, using a pledget of cotton-wool for chicks and a brush for adults, and part the down or feathers along the middle line. Using blunt-nosed scissors and forceps, slit the skin along the mid-line from the vent to well up the neck and along each leg, reflect it on each side, and note any bruising or injury, or if the carcase appears fevered, anæmic, etc. Pick up the loose abdominal wall on the left side (right side as the birds lie) high up the body to avoid damaging the intestines, and cut through with scissors along the level of the cartilaginous junctions of the ribs with the keel bone, continue the cut back across the abdomen and cut through the ribs at these cartilaginous junctions on each side. Sever the pleura and the pectoral muscles with a scalpel, grip the end of the keel bone with forceps and cut the coracoid and clavicle bones on each side with bone forceps, lift the keel bone and cut free. Trim off excess skin and fat over the abdomen.

(2) *Examination of Organs in situ.*

While all organs are undisturbed the bird is examined by the pathologist, who may direct the lines along which he wishes further examination to proceed. The following lesions are commonly observed (infectious diseases are grouped together in approximate order of incidence):

Tuberculosis in the form of multiple tubercles involving the liver, spleen or intestines.

Blackhead, a disease commonly affecting turkeys, caused by the protozoan parasite *Histomonas meleagridis,* and diagnosed by the characteristic concentrically formed, slightly depressed lesions in the liver and a typical necrotic typhlitis.

Aspergillosis, caused by the mould *Aspergillus fumigatus,* and occurring as a cheesy thickening on the inside of the air-sacs with possibly some green or brownish sporing mould growth. The lungs may also show nodular lesions, and in chicks similar yellow nodules often occur on the inside of the keel bone.

Multiple necrosis in the form of varying sized nodular lesions involving the internal organs, and often accompanied by a *necrotic enteritis. F. necrosis* and clostridium types are most commonly associated with this condition, which may need to be differentiated from tuberculosis.

The following are diseases of unknown or uncertain etiology which give rise to marked lesions as described:

Tumour formation involving one or more organs. Varied types of tumours occur in individual birds; common ones are sarcomas, carcinomas, and a fibromatous type.

Leukæmia causing hypertrophy of the liver, spleen or kidneys. The liver may attain an enormous size in this disease before the bird succumbs.

Egg peritonitis, varying from the acute condition, in which an active peritonitis is accompanied by the presence of much egg material free in the abdominal cavity, to the chronic form, in which only a little inspissated egg material may be present.

Visceral gout, characterised by white deposits of uric acid in the urinary system, and on the surface of any or all of the internal organs or their membranes. A deposit often occurs inside the pericardial sac.

Other more general and less obvious lesions to note are: Bronze-coloured liver, suggesting *fowl typhoid;* miliary lesions in the heart, liver or spleen, suggestive of bacillary *white diarrhœa* or other bacterial infections; general congestion and petechial hæmorrhages around the coronary groove of the heart, associated with acute bacterial disease such as *fowl cholera,* or with certain of the virus diseases, e.g., *infectious laryngo-tracheitis* and *fowl pest.* Look for any impaction of the intestines, etc., *injury* to any part; and, finally, any enlargement of the plexus of nerves supplying the gizzard and intestines and suggestive of *Neurolymphamotosis.*

Chicks may show a distended gall bladder, with pneumonia and general congestion accompanied by a nasal discharge, all of which suggests *chill,* a well-recognised condition occurring in young chicks exposed to adverse conditions. Also in chicks, typical yellow or white nodules in the lungs, liver and heart muscle, with an unabsorbed yolk-sac, are seen in cases of *bacillary white diarrhœa;* and the cæca distended with blood caused by *coccidiosis.* A thickening of the ribs with folding of the cartilaginous junctions suggests *rickets,* which may be confirmed by the finding of a rubbery condition of the mandibles and long bones of the limbs. *Beak disease* is a condition in which the points of the beak, particularly of the lower mandible, become necrotic, and portions slough off as a result of mixed bacterial infection, often with *Cl. welchii* and staphylococci.

Turkey poults may show a severe pericarditis with a considerable cheesy exudate, due to infection with a *Salmonella* species of bacterium, usually *S. ærtrycke.* American workers have named this condition *epiornithological pneumo-pericarditis.*

(3) *Examination for Internal Parasites.*

Without disturbing the other organs unduly, cut off the duodenum close to the gizzard and draw out all the intestines. Note any abnormality, severe inflammatory conditions, etc. Open up the intestines, note any enteritis, and examine for parasites as follows:—

Large roundworms (*Ascaridia*).—These are obvious to the naked eye, the males measuring 50-76 mm. and females 72-116 mm. in length. Unless these

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parasites are very numerous or causing blockage of the intestine, they are not of primary importance. The eggs, which may be found in material examined microscopically, for other parasites are oval, unsegmented, measuring 80μ-85μ by 43μ-50μ.

To examine for other parasites, scrape the intestine in two widely separated areas with a scalpel, transfer a little of the scrapings to a slide, mix with a drop of water, cover with a $\frac{3}{8}$-in. square coverslip, and examine systematically with the $\frac{2}{3}$ objective for:

(a) *Small roundworms* (*Capillaria retusa* and other *Capillaria* spp.). Males 9.5-11.5 mm. and females 10.5-14 mm. long; eggs are yellow, oval, unsegmented, with a double operculum, and measuring 48μ-53μ by 24μ. The numbers of these parasites present may be assessed by fishing in the intestinal contents with a dissecting needle. It may be noted that the finding of a large concentration of eggs in the wet preparation described can be due to the presence of a gravid female at or near the site of a scraping, and may not itself indicate a heavy infestation.

(b) *Tapeworms* (*Davainea proglottina* and other *Davainea* spp.). The segments of these worms vary in size, but are usually too small to be distinguished by the naked eye, and rarely occur in lengths containing more than five proglottides in preparations made as described above. The size of the proglottides found varies, the largest may take up half the microscope field when using the $\frac{2}{3}$ objective. Members of the species have a scolex with an armed sucker behind this, which causes considerable irritation. The hooks are hammer-shaped and, on the rostellum, are 7.8μ long. The scolex remains attached to the intestinal mucous membrane for long periods, and the ripe segments become detached and are passed out in the fæces. These segments may be visible to the naked eye if present in large numbers; under these conditions careful observation and the use of a hand lens will distinguish them from food particles, particularly if they exhibit movement, as in the case of a freshly killed bird. In the intestines up to nine proglottides may be found attached together, the whole specimen measuring 0.5-3 mm. in length.

The large tapeworms (*Raillietina tetragona* and *R. echinobothrida*) may measure up to 25 cm. in length, with a thin "neck" and a smaller scolex with minute hooks and an oval sucker. These parasites are relatively uncommon in this country.

(c) *The Oocyst stage of Coccidia* (*Eimeria avium and other spp.*). These will almost always require to be examined under the $\frac{1}{6}$ objective after a preliminary search with the $\frac{2}{3}$ objective, and in some cases are impossible to find except under the higher magnification. They are double-walled with granular contents, are usually egg-shaped but may appear circular, and vary in size from 9μ by 10.5μ to 29μ by 38μ.

Coccidiosis is an important and widespread disease of poultry. The finding of comparatively few oocysts, particularly if a marked enteritis is present, or if the intestines are flaccid and very anæmic in appearance, and there is evidence of poor condition, together with a history of toxic symptoms, cannot be disregarded, as there is a tendency in the chronic stage of the disease for the oocysts to be given off in batches from the parasitised cells of the intestinal mucous

membrane. In a suspected case more than one preparation of scrapings from the intestines should be examined. In chicks and young birds the cæca are the parts usually affected, and are always examined for the presence of oocysts.

Parasites occurring in other situations of the body are:—

Crop worms (*Capillaria contorta*). These occur in the crop and œsophagus, particularly in ducks, and may cause distension of the crop with pressure on the vagus nerve. The male worm measures 12-17 mm. and the female 27-38 mm. in length, eggs are typical of the species. The worms may be found by fishing with a dissecting needle in the crop contents.

Gizzard worms (*Amidostomum nodulosom*). The species commonly affects geese, and is a small nematode, males measuring 10-17 mm. and females 12-24 mm. in length. The eggs are segmented, measuring 66μ by 100μ. The parasite is often a reddish-brown colour. To detect these worms in geese, remove and cut open the gizzard, remove the contents carefully, then place the everted organ in a bowl of water and wash off gently. The parasites may be seen protruding from the cracks they cause in the horny lining. Remove specimens to a watch glass and examine under the $\frac{2}{3}$ objective.

Gapeworms occur in the trachea and are dealt with under section 5, "Completion of Post-mortem Examination."

Air-sac mites (*Cytoleichus nudus*). These are small mites living in the air-sacs and occasionally the lungs. They are yellowish in colour and have four pairs of suckered legs. The lining membranes of the main air-sacs may be found on each side adjacent to the pelvis and kidneys, also overlying the plexus of nerves and blood vessels supplying the wings, where these leave the body; the membranes are thin, transparent, and very readily damaged. Sometimes these membranes show a number of small fatty particles, which may lead to a suspicion of air-sac mites being present. Remove suspected material, with, if necessary, a small portion of membrane, from the air-sac membranes and examine under the $\frac{2}{3}$ objective.

(4) *Bacteriological Examination* (*Primary*).

Lesions suggestive of bacterial disease will already have been noted, but a bacteriological examination should be a routine procedure, as lesions may be absent or only slight and indefinite.

With care it will be possible to carry the examination to this stage without disturbing any organ other than the intestines. Except where botulism or other anærobic infection is suspected, ærobic cultures only are necessary. These are made from the liver, as being the largest conveniently situated organ of the body, and the one likely to be heavily infected in any bacterial disease which has resulted in the death of the bird. Other situations from which cultures are often made are: the heart blood, the blood serving at the same time to enrich the culture medium in this case; and the bone-marrow, this being used on occasions when material is not fresh, as cultures from this situation are less likely to be contaminated by post-mortem invaders. Cultures from special situations, e.g., glands, etc., are not used to any extent in poultry.

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Cultures are made on nutrient agar slopes, as follows:—

Liver: Sear the surface of the organ on the right lobe, introduce a flamed nichrome wire loop about 1½ in. long through the seared area, revolve a few times, withdraw, and inoculate the slope in the usual way.

Heart blood: Open the pericardial sac. Sear the surface of the right ventricle of the heart, grip the apex with forceps, pass the tip of a sterile pasteur pipette through the seared area, and, using a rubber teat, draw up the available quantity of blood; an appreciable quantity can usually be obtained, even from quite young chicks, in this way. Inoculate the medium first, then deliver a drop of blood on to two slides and spread these in the appropriate manner for subsequent staining.

Bone-marrow: Strip the muscle away from the tibia, cut off at the tibio-tarsal (hock) joint, detach the foot from the board and use this for holding. Flame the naked bone and cut across with sterile bone forceps; the inoculum is then accessible.

Blood films are stained with Leishman's stain. If taken from birds which have died of *fowl cholera*, such films show very numerous small bacillary forms with the poles more deeply stained than the centre. In other bacterial diseases organisms may be very scanty in the blood. *Sleeping disease* is characterised by the occurrence of chains of encapsulated streptococci, and *spirochætosis* by the presence of *Spirochæta gallinæ* in the blood.

In cases of suspected *aspergillosis*, if a sporing mould growth is present a little of this is examined under a coverslip in lactophenol. The typical Aspergillus morphology (sunray effect), with the spores arranged in a rounded mass at the ends of radiating hyphæ, may be found, using the ⅔ objective. A culture may be made on malt agar. In chicks, where obvious lesions may be absent, cultures are taken from the lung tissue after searing the surface.

Botulism or *limberneck* is the only disease of importance caused by anærobic bacteria. A severe enteritis, with a history of twisted carriage of the head and evidence of the birds having had access to rotting material (often fruit), may suggest this disease. Filtrates of intestinal material may be tested for toxicity. Cultures are attempted from the intestinal material, and also from any suspected food. These are done in Robertson's meat broth heated to 60 deg. C. for 30 minutes after inoculation.

Joint infections. Poultry, particularly geese and ducks, are liable to infectious joint trouble, in which the birds show swollen joints. From the excessive amount of synovial fluid contained in such joints cultures are obtained. The commonest joint and the one most easily accessible for this purpose is the tibio-tarsal (hock) joint. To obtain culture material, release the foot from the board, bend the joint to stretch the skin over the posterior area, sear this, and insert a sterile paste pipette and withdraw some fluid. Inoculate a nutrient agar slope and expel a drop on to a slide; from this a film is prepared and stained by Gram's method. Staphylococci are usually found. The condition is well recognised in geese as *infectious osteo-arthritis*.

Bumble foot may result from a variety of infections, often mixed. Smears from the lesions may be examined, particularly for acid-fast organisms.

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Tuberculosis. Diagnosis is made by crushing a small lesion (older lesions may contain less viable organisms, which are not likely to stain well) and preparing a smear from this. Stain by Ziehl-Neelsen's method and examine. No difficulty is experienced in finding the bacilli, which are very numerous in smears made from lesions in poultry. For the cultivation of the avian strain of *Myco. tuberculosis,* Lowenstein-Jensen's medium is excellent. Cultures may be made direct from lesions, growth occurs in two to four weeks, and is rather moist as compared with other strains.

Clostridium welchii infections have been noted in poultry on rare occasions. Appropriate culture methods are used.

(5) *Completion of Post-mortem Examination.*

Lift the gizzard clear, sever the membranes, and turn this organ together with the liver and spleen over the right ribs. Examine the nerve supply carefully for any enlargement suggestive of *Neurolymphomatosis.* Examine the kidneys for any abnormality, particularly for abscess formation found in *avitaminosis "A"* (which is confirmed by later examination of the pharynx), and for lesions of *tuberculosis,* which may involve the hip joint, causing lameness.

Next observe the ovary, a lymphomatous condition is typically associated with *neurolymphomatosis;* atrophic ("blighted") yolk sacs may be present, indicative possibly of the carrier stage of *bacillary white diarrhœa,* or of other bacterial disease; and *cystic ovaries* are sometimes found.

The kidneys are lobulated and extend from the ribs to the pelvic bones, being closely adherent to depressions in the skeletal framework. With scissors, slit along a line dividing the kidneys and dissect out these organs, cutting associated blood vessels, but taking care not to damage the sacral plexi of nerves which pass through the substance of the kidneys. These nerves and ganglia are examined for any swelling which may be caused by *neurolymphomatosis;* this examination is carried on down the femoral nerves between the leg muscles as far as the first joint. Examine the spine along the lower ribs and lumbar region for any fracture caused by a blow or by crushing, as, for instance, by the bird being run over by a vehicle.

Examine the brachial plexi of nerves for *neurolymphomatosis* by removing the air-sacs and blood vessels overlying these. Impaction of the crop may lead to a suspicion of involvement of its nerve supply; in this case dissect away the crop near its junction with the proventriculus (stomach) and continue the dissection carefully beyond the crop and on either side of the œsophagus, so as to expose the nerve supply.

Portions of any nerve suspected of being affected with *neurolymphomatosis* are fixed in formol-saline; if it is desired, confirm the diagnosis by the examination of sections. Gross macroscopic lesions of the nerves are, however, usually considered diagnostic.

The gizzard and proventriculus may now be removed together and slit open. Note any impaction, absence of grit, or presence of any foreign body, e.g., pieces of wire. The contents are removed, the organ washed out, and the lining membrane examined for evidence of erosion due to some irritant poison. *Phosphorus poisoning,* sometimes due to an intake of rat poison, is detected by

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the peculiar smell and by the presence of whitish fumes, on first opening the gizzard, caused by oxidation of the free phosphorus; this fuming is fugitive. If poisoning is suspected, material to include portions of gizzard lining, gizzard and crop contents, liver, kidney, and intestine is collected.

Examine the lungs for pneumonia, and particularly for severe pneumonia or congestion accompanied by a serous effusion, which occurs in cases of *infectious laryngo-tracheitis;* also examine for lesions of *aspergillosis. Advanced pulmonary tuberculosis* is rare amongst poultry.

Finally, open the beak and cut the mandibles apart, continuing the cut down one side of the neck to expose the larynx. Examine the throat and palate for diphtheritic lesions suggesting *roup* or *fowl pox*, and for white nodular lesions typical of *avitaminosis "A"* (accompanied by abscess formation in the kidneys). Observe any nasal catarrh. Grip the larynx firmly with clean forceps and slit open slightly with clean scissors; an intense hæmorrhagic tracheitis, which may be accompanied by cheesy deposits and heavy catarrh, is found in cases of *infectious laryngo-tracheitis.* If other lesions and the history of the outbreak suggest this, the whole of the larynx with the unopened trachea is removed for further examination by the inoculation of material into healthy birds. If *infectious laryngo-tracheitis* is not suspected, slit open the trachea and observe any mechanical blockage or any cheesy deposits caused by *roup* or *fowl pox.*

Gapeworms (*Syngamus trachea*) are examined for at this stage. They are a comparatively rare finding in domestic poultry kept under modern conditions. When they do occur they will be found in the trachea, which must be split along its whole length. The worms are typically found in copula, the shorter male attached near one end of the female, giving a Y formation. This is a nematode parasite, the males measure 2-6 mm. and the females 5-20 mm. in length.

It it is suspected that *neurolymphomatosis* is affecting the optic nerve, this must be carefully dissected out. Open the skull along the cranial mid-line, remove the nerve with a portion of eye to facilitate handling, and place in fixative for sections to be cut.

(6) *Specific Laboratory Techniques.*

Bacteriological Procedure.—Media required are as follows:—

Nutrient Agar —Lemco or infusion agar, pH 7.3.
Nutrient Broth —Lemco or infusion broth, pH 7.3.
Robertson's Meat Broth—pH 7.6.
Malt Agar —3.5 per cent. Allen & Hanbury malt extract and 2 per cent. agar powder, sterilise at 10 lb. for 20 mins. No filtration or pH adjustment necessary.
Fermentation Test Media—Peptone water pH 7.6, with 1 per cent. fermentable substance and 1 per cent. Andrade's Indicator.
Peptone Water —pH 7.3, for Indol tests.
Nutrient Gelatin.
Litmus Milk.

It is also useful to keep a supply of McConkey's agar in screw-capped bottles, to which the neutral red is added immediately before pouring plates, so that mixed cultures suspected of including a *Salmonella* spp. pathogen may be dealt with.

Agar slopes inoculated from birds are incubated for 24 hours and examined. Any showing no obvious growth are incubated for a further 24 hours before being discarded. Films are prepared from cultures which have grown, and stained and examined in the usual way.

The following are the common causative organisms of the various bacterial diseases in poultry :—

Salmonella pullorum	—Bacillary white diarrhœa.
„ *gallinarum*	—Fowl typhoid.
„ *œrtrycke*	—Paratyphoid disease.
„ *enteritidis*	—Paratyphoid disease.
„ *anatis*	—Keel of ducklings.
Pasteurella avisepticus	—Fowl cholera.
Staphylococci	—Infectious osteo-arthritis.
Listerella spp.	—Listerellosis (rôle not fully established).
Escherichia coli	—Colibacillosis, and post-mortem invaders.
Proteus morganii	—May infect weakly chicks, also post-mortem invader.
Streptococcus gallinarum	—Sleeping disease.
„ *fœcalis*	—Post-mortem invader.
Clostridium botulinus	—Limberneck (botulism).
„ *welchii*	—Very rare anærobic infection.

The list does not exclude the possibility of other organisms being isolated. Fermentation and slide agglutination tests are used to identify the organisms obtained. In the case of *Pasteurella avisepticus*, a suspected culture is tested by intravenous inoculation into a rabbit and a fowl, and should cause death with, in the fowl, typical symptoms and lesions, and yield a typical blood film after death.

Table I gives a rough outline of procedure for routine work.

TABLE I.

Colony Types and Gram Films.

Irregular white or greyish colonies. Short thick Gram negative rods.	Entire white or yellow colonies. Large clusters of Gram positive cocci.	Colourless or greyish colonies may be discrete; fluorescent, luxuriant growth. Small Gram negative filamentous rods.	Discrete colourless colonies, may be scattered. Gram positive slender rods. Often in palisades.	Discrete colourless or translucent colonies. Chains or pairs. Gram positive cocci.	Discrete greyish or white colonies. Growth may be best from blood. Very small Gram negative bipolar rods.
Coliform, etc. If desired to identify, inoc. sugars, otherwise inoc. lactose only.	*Staphylococci* Inoc. gelatin and await pigment development. Test further if desired.	*Salmonella* Inoc. sugars. Put up agglutination tests. Test motility.	*Listerella* Inoc. sugars. Test motility.	*Streptococci* Inoc. blood agar plate, and sugars, etc.	*Pasteurella* Inoc. sugars and experimental animals.

Table II gives differential tests for organisms of the *Salmonella* group which may be isolated.

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TABLE II.

Differential Characters of Salmonella Spp.

	Sugar fermentations				Agglutination of specific antisera		
	Lactose	Maltose	Mannite	Glucose	Pullorum	Aærtrycke	Enteritidis
Salmonella pullorum	—	—	A	A	+	—	+
„ gallinarum	—	A	A	A	+	—	+
„ ærtrycke	—	AG	AG	AG	—	+	—
„ enteritidis	—	AG	AG	AG	+	—	+
„ morganii	—	V	—	AG	—	—	—

In the most recent (fifth) edition of the Manual of Determinative Bacteriology by D. H. Bergey, the causal organism of fowl typhoid has been removed from the genus *Salmonella,* to be called *Shigella gallinarum;* the merits of this change are open to question, and for the sake of simplicity the older generic name is used throughout this article. Both *S. pullorum* and *S. gallinarum* are sharply defined from the other members of the genus by being non-motile. Experience has shown that these organisms rarely form gas from the " sugars " on first isolation, though occasionally several gas-forming strains will occur in rapid succession. An attempt was made particularly by the early German workers to separate *S. pullorum* into types "A" and " B " on the basis of gas production in the " sugars," but this never came into general use. *S. gallinarum* ferments dulcite comparatively slowly, 48 hours or more incubation is required. The two organisms are antigenically homologous. Slide agglutination tests on primary cultures, together with fermentation tests give a rapid and precise method of identifying the organisms. Difficulty is sometimes experienced with other *Salmonella* species—*S. ærtrycke,* in particular, may fail to give clear-cut agglutination readings on slides, although no obvious colony variation is apparent. It may be noted that both *S. pullorum* and *S. gallinarum* possess the somatic antigen which is also common to *S. enteritidis* and *Eberthella typhosus. S. ærtrycke* and *S. enteritidis* are readily differentiated by fermentation of inosite. The commonest of these two organisms in natural outbreaks is *S. ærtrycke,* which gives rise to an increasing amount of trouble in poultry flocks; the infection being passed on through the egg by infected hens, in the same way that *S. pullorum* is, so that chicks are already infected when they hatch. A supply of fresh native antisera is usually available in a laboratory undertaking poultry work, since this will include flock blood tests to detect adult carriers of *Salmonella* infections.

With regard to coliform types, it will depend on the individual cases whether or not it is necessary to type these specially. Their exact significance is sometimes obscure, and they may well be the result of post-mortem invasion, particularly if carcases are showing signs of post-mortem change when examined. In such cases, however, *Streptococcus fæcalis* often grows in conjunction with them. Obviously, if several birds, particularly chicks, from a single disease outbreak have yielded apparently similar cultures, identification of such cultures is more necessary. Coliform types are often obtained from chicks suffering from the entity recognised under the name of *chill.*

Streptococcal septicæmia is reported as occurring, particularly in the United States. No cases were ever encountered during the time in which the work was done upon which this article is based.

Pasteurella avisepticus, the causative organism of *fowl cholera,* is a member

of the group showing true polar staining (not to be confused with the staining of polar granules shown by the Corynebacteria), when stained with Leishman's stain, dilute carbol-fuchsin, or with methylene blue. It is non-motile. A capsule may be demonstrated by relief methods. A pathogenicity test, as described, is used in examining cultures.

Staphylococci are identified by appropriate methods. Cultures obtained from affected joints may be strongly hæmolytic. *Staph. aureus* is the organism usually present; the condition may become generalised and fatal, when the organisms are recoverable from the blood and internal organs.

Listerella species. In 1937 Paterson reported the occurrence of Listerella infection in fowls in East Anglia, and very shortly afterwards strains were isolated from fowls in the South-West area. Since then this organism has been repeatedly isolated from poultry specimens during the routine bacteriological examination. The organism is a small motile, Gram positive bacillus, and is apparently not quite identical with the single recognised member of this genus, *L. monocytogenes* (Murray, Swan and Webb). Its significance is not yet clearly defined; it has been found in conjunction with a variety of other disease conditions, as well as in uncomplicated cases. It was thought to give rise to nervous symptoms, in particular to an abnormal carriage of the head with the neck twisted downwards, but such symptoms are also associated with intense toxæmia which may arise in cases of chronic parasitism, e.g., by coccidia. The only typical post-mortem lesion—a massive endocarditis—was seen only in a proportion of cases.

The fermentation reactions, based on strains isolated from cases in the South-West area, are as follows:—

Lactose: Not fermented.

Maltose: Acid formed, no gas.

Mannite: Not fermented.

Saccharose: Acid formed, no gas.

Salicin: Acid formed, no gas.

Litmus milk: Reduction of litmus in the lower portion of the medium, with sharp line of demarcation.

Indol formation: None.

These reactions may be subject to slight variation and are apparent after 24-48 hours' incubation. The reaction in litmus milk is typical. Motility is never very marked, and in some strains is scarcely discernible; it is usually greater in cultures which have been incubated at 22 deg. C.

Agglutination tests, using "H" and "O" antigens, have been developed, and have shown an erratic, and sometimes high, proportion of low titre reactions when blood samples from a flock in which the infection is known to exist are tested; there is poor correlation with specific losses.

Cl. botulinus. A toxicity test done on bowel filtrates is the most direct method of determining this condition. Cultures may be attempted from the intestine, and also from suspected food material. Primary cultures in Robertson's meat broth, which were heated at 60 deg. C. for 30 minutes prior to incubation, may be partly purified by repeated heated subculture, and if organisms morphologically

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TABLE III.

Fermentation Reactions of Gram Negative Aerobic Bacilli from Poultry.

	Lactose	Maltose	Dulcite	Lævulose	Mannite	Glucose	Saccharose	Salicin	Xylose	Inosite	Litmus Milk	Indol	Motility
E. coli communis	AG	AG	AG	AG	AG	AG	—	AG	AG	—	AC	+	+
E. coli communior	AG	AG	AG	AG	AG	AG	AG	—	AG	—	AC	+	+
E. neapolitana ...	AG	AG	V	AG	AG	AG	AG	AG	AG	—	AC	+	—
E. acidi lactici ...	AG	AG	V	AG	AG	AG	—	—	AG	—	AC	+	—
S. pullorum ...	—	—	—	A	A	A	—	—	—	—	A-AIK	—	—
S. gallinarum ...	—	A	A	A	A	A	—	—	A	?	A-AIK	—	—
S. ærtrycke ...	—	AG	AG	AG	AG	AG	—	—	AG	AG	A-AIK	V	+
S. enteriditis ...	—	AH	AG	AG	AG	AG	—	—	AG	—	A-AIK	—	+
P. morganii ...	—	V	—	AG	—	AG	—	—	V	—	AIK or V	+	V
S. anatis	—	AG	AG	AG	AG	AG	—	—	AG	—	A-AIK	—	+
Past. avisepticus	—	V	—	A	A	A	A	—	—	?	—	V	—

like *Cl. botulinus* are present anærobic plate cultures may be attempted from the partly purified broth cultures. Type "C" is the strain usually affecting poultry.

To test for toxins, as much material as is available is collected from the intestine, diluted with an equal volume of saline, and centrifuged at 3,000 r.p.m. for 30 minutes. Decant, and pass the supernatant fluid through a bacteriological filter. Graded doses of this filtrate from 0.5 c.c. downward are inoculated intravenously into mice, and, if toxic, then toxin-antitoxin titrations are carried out.

Aspergillum fumigatum gives a good growth on malt agar in 24-48 hours at 37 deg. C. This rapid growth at what is, for a mould, a high temperature is specific for the pathogenic strains. The growth is of a green colour in the sporing stage, and should be examined in lactophenol for the typical fruiting head already described. Suspected litter or food may be examined by plating a little on a malt agar in petri plates and incubating at 37 deg. C. for 48 hours.

Table III shows some reactions of the Gram negative ærobic bacilli commonly met with.

Virus Diseases.—Fowl pox and *roup* are usually diagnosed by the lesions produced, and in many cases examination of the flock will be sufficient. Material from lesions may be tested by scarification of the comb or wattles of a healthy bird, and rubbing material into the scarified area.

Newcastle disease and *fowl plague* or *pest,* and *infectious laryngo-tracheitis* are important virus diseases, the former two being notifiable under the Diseases of Animals Act, and diagnosis of all of them has to be made by the Ministry of Agriculture Laboratory at Weybridge. The method consists essentially in inoculating healthy birds with material from suspected cases. In the case of *infectious laryngo-tracheitis,* as has already been described, the whole trachea and larynx are removed and despatched in a sterile container to the Ministry's laboratory, where material from them is inoculated into the trachea of a healthy bird. In the case of fowl pest (this name covers also Newcastle disease and fowl plague), a whole carcase is required by the authorities who carry out inoculation tests. Lesions, symptoms, history, and the absence of any other positive finding are all suggestive. It is stated that 0.0000000001 c.c. of the whole blood from an affected bird is infective by subcutaneous inoculation, and 0.01 c.c. given by the mouth. This disease entity is highly infectious and rapidly fatal.

Parasitological Procedure.—Apart from the methods given for macroscopical and microscopical examination of intestinal contents, air-sacs, trachea, legs and feathers, no special techniques are necessary. The precise identification of nematodes, cestodes, etc., is highly specialised work and not essential to a diagnosis.

For the preservation of nematodes, use 2 per cent. formalin in 70 per cent. alcohol; for cestodes, use either the same or 10 per cent. formalin. Fleas, lice and mites may be placed in 50 per cent. alcohol, and later transferred to absolute alcohol.

Histoiogical Procedure.—Sections are prepared, when necessary, by the usual techniques. The diagnosis of neurolymphomatosis often necessitates the cutting of transverse sections, and these are conveniently and rapidly prepared with a CO_2 freezing microtome; the tissue is fixed overnight in formol-saline, and sections 8-10μ thick cut. Drop the sections into water and, manipulating with a drawn-out

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glass rod, stain with hæmatoxylin and eosin, wash after the counter-stain and transfer direct to a slide. Blot and cover with origanum oil to dehydrate and clear, drain and mount. In affected cases the spaces between the nerve fibres are infiltrated by lymphocytes and degeneration of the fibre occurs.

A rapid method for the examination of suspected lymphomatous nerves is to make smears from a freshly cut surface of the nerve and stain with Leishman's stain, and examine for the presence of lymphocytes.

Examination for Common Irritant Poisons.—Material is collected as already described, in two parts. The total weight should be about 20 gms. and the exact figure should be noted. That portion of material consisting of gizzard lining, liver, kidney, crop contents and intestine with contents is cut up and transferred to a 300 c.c. pyrex kjeldahl flask (do not include any grit, as this causes severe bumping). Add about 10-15 gms. potassium sulphate, a pinch of powdered selenium, and 25-50 c.c. concentrated sulphuric acid, according to the bulk of the material. Shake.

The other portion of material, consisting of gizzard contents with grit, is placed in a 100 c.c. pyrex beaker, a few grammes of potassium sulphate added, and the contents covered with concentrated sulphuric acid.

Heat both flask and beaker in a fume cupboard, proceeding cautiously at first, and increasing the heat when excessive frothing ceases; the addition of a small pellet of *pure* paraffin wax cuts down frothing. When the organic material in the beaker goes into solution, add the fluid portion of its contents to the flask, wash out with hot concentrated sulphuric acid and transfer the washings. Now boil the material in the flask over a naked flame until a more or less clear and slightly reddish solution is obtained; this may take several hours. Allow to cool thoroughly.

When cold, dilute cautiously with distilled water up to about 100 c.c., cool under the tap if necessary, and transfer to a 250 c.c. graduated flask. Wash out with three changes of distilled water and transfer the washings. Make to the mark, cork and mix. This solution will contain all the metallic poisons in solution, except lead, which may be present as a white precipitate.

The common metallic poisons found are arsenic, copper, mercury and lead.

First examine for arsenic by the Gutzeit test: A 250 c.c. conical flask is fitted with an I.R. bung carrying a special Gutzeit tube, which is a glass tube open at both ends and having a hole blown in the side at the commencement of the tapered end which is inside the flask. Into the upper (wide) end of the tube insert a rolled-up 9 cm. filter paper which has been soaked in 10 per cent. lead acetate solution and dried, and over the upper end place a 3 cm. filter paper which has been soaked in 10 per cent. mercuric chloride solution and dried; secure this in place with a rubber band (special Gutzeit tubes having an arrangement of ground glass surfaces to hold this paper in place can be purchased). Set up this apparatus and place in the flask a few pieces of granulated zinc. Add quickly 100 c.c. of the sulphuric acid solution of suspected material and replace the bung and fitting *immediately*. Place on a warm surface for 2-3 hours. The presence of arsenic is shown by a yellow stain on the mercuric chloride paper, deepening to brown or black if much is present.

Standard papers may be prepared, using solutions of arsenious oxide of known strength; papers representing 0.0001 gm., 0.002 gm., 0.005 gm., and 0.001 gm. are useful. These must be freshly prepared.

During the process of digestion, some of the arsenic present may become oxidised, and in this form will not be liberated until reduced to the arsenious form. If it is desired to make a very careful estimation this reduction may be carried out by adding a little sodium sulphite to the solution before testing, and boiling gently under a reflux condenser for a few minutes, until no more sulphur dioxide is given off.

To examine for lead: Filter off any white precipitate from the sulphuric acid solution. Wash with distilled water. Keep the filtrate for other examination, discard the washings. Now wash with concentrated ammonium acetate solution, collecting the washings separately. Any lead sulphate present will be dissolved and pass through the filter paper. To this filtrate add a few drops of potassium chromate solution, a deep yellow colour becoming a precipitate in heavily plumbicised cases indicates the presence of *lead*.

To examine for other metallic poisons: Filter some of the sulphuric acid solution. Make the filtrate just alkaline with ammonia.

At this stage a blue precipitate, redissolving to form an azure-blue solution, indicates the presence of *copper*.

Divide the alkaline solution into two parts. Acidify one part with hydrochloric acid, cool and pass sulphuretted hydrogen through for a few minutes:—

Yellow	precipitate	indicates	selenium, sulphur or arsenic.
Lemon	„	„	cadmium or arsenic.
Black	„	„	copper, mercury or bismuth.
Orange	„	„	antimony.
Dark brown	„	„	tin.

Arsenic will have been confirmed by the Gutzeit test.

Copper—the blue colour with ammonia already described.

Mercury—to confirm take a fresh portion of filtrate, render alkaline with ammonia, and then acid with hydrochloric acid. Add a little saturated stannous chloride solution; a white precipitate is confirmatory.

All chemicals used should be of ANALAR Standard. For the Gutzeit test, materials must be arsenic-free, specially designated chemicals are supplied for this test by the British Drug Houses, known as AsT Standards.

Phosphorus: The presence of free phosphorus may be confirmed in the following manner:—

A flask of about 500 c.c. capacity is fitted with an I.R. bung carrying an air condenser in the form of a long tube bent at right angles near one end, the short arm passed through the I.R. bung. The flask contains water to which a few drops of hydrochloric acid has been added. The whole apparatus is transferred to a perfectly dark room, a little of the suspected material is added to the flask, which is then heated over a shielded flame. If free phosphorus is present as the liquid becomes hot a luminous ring will form and travel along the air condenser. If much phosphorus is present, several rings will form. The effect must be carefully watched for in perfect darkness and is soon past.

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Summary

An account of techniques adopted in the routine examination of poultry cadavers in order to establish the nature of disease outbreaks is given under six headings as follows:—

(1) Preparation, examination for External Parasites, and opening of carcase.

(2) Examination of organs *in situ*.

(3) Examination for Internal Parasites.

(4) Bacteriological Examination (primary).

(5) Completion of Post-mortem Examination.

(6) Specific Laboratory Techniques.

The various external parasites are given with the stage in the examination at which they are usually obvious. External lesions are noted, and the method adopted for opening the carcase is described.

Outstanding lesions to be expected for each disease are indicated, the infectious diseases being grouped together, followed by diseases of uncertain etiology or due to physiological disturbance, all taken roughly in order of importance. A note of general lesions common to several conditions follows, and finally lesions to be found in chicks are detailed. It may be noted that more immediately obvious lesions are not necessarily those associated with the more important diseases.

Internal parasites include protozoa, helminths and one arthropod. The protozoa and helminth parasites are all primarily inhabitants of the alimentary tract (with the exception of the *gapeworm*, which is dealt with separately), the most important single parasitic desease being *coccidiosis*, caused by a protozoa. The single arthropod parasite is the *air-sac mite*. Precise situations to examine are given and the simple method (by a wet preparation on a microscope slide) to recognise the parasites is described.

Primary bacteriological examination: The liver is recommended as the organ of choice for this, alternatively the heart blood, and in birds showing post-mortem change the bone-marrow are cultured from. If infectious osteo-arthritis is suspected, a culture is taken from a joint. Both the mycoid infection *aspergillosis* and *tuberculosis* are dealt with by means of direct microscopical examination.

Further examination of the carcase is as follows: The main nerve plexi are exposed to detect lesions of *fowl paralysis*; this is done by dissecting away the kidneys, also the tissues overlying the brachial plexi. Then the mouth, pharynx and larynx are laid open; at this stage the nematode parasite causing *gapes* is looked for in the trachea. Finally, the gizzard is split open to ascertain whether there is any evidence of irritant poisons having been ingested.

Dealing with further laboratory procedure, a description of minimal requirements for recognising the bacteria pathogenic to fowls is given with all necessary culture tests, and list of organisms likely to be encountered, including contaminants and those whose status is uncertain. With the exception of *Cl. botulinus*, all the poultry pathogens are ærobic, growing readily on simple media, and neither enriched nor selective media are usually required. Three tables of characters

are included. Virus infections are described; apart from *fowl pox* and *roup*, which may be diagnosed on lesions, the virus diseases of poultry are notifiable, which means that a diagnosis has to be made by the Ministry of Agriculture.

Brief notes on the preservation of parasites and on histological procedures are included, and there is a complete description of methods adopted for recognising the common metallic poisons and phosphorus.

Acknowledgements

I am indebted to Mr. C. V. Watkins, M.R.C.V.S., D.V.S.M., who was my chief during the time that the material for this paper was collected, for help and guidance; and to Mr. J. Francis, B.Sc., M.R.C.V.S., for encouragement and advice in the preparation of the manuscript.

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CLINICAL ARTICLE

CASE OF EQUINE ENCEPHALOMYELITIS FROM SYRIA

By I. H. PATTISON

Government Veterinary Laboratory, Tel-Aviv, Palestine.

Introduction

In a previous paper (Pattison, 1945) histological evidence was presented indicating that a form of equine encephalomyelitis occurs in Palestine, and some attempt was made to classify the disease in relation to forms of equine encephalomyelitis known to exist in other parts of the world.

This paper is a brief report on a case of equine encephalomyelitis from the neighbouring territory of Syria.

The Disease in Syria

The disease, now definitely known to be a form of equine encephalomyelitis, is believed to have existed in at least one area of Syria for some considerable time. The area in question is that adjacent to the most northerly frontier of Palestine, with Metulla and Banias localities in which the disease has frequently been observed. French authorities have declared that they never could keep horses

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in Banias because of the prevalence of the disease, and during the Syrian campaign of World War II mounted troops in the area, when passing through Banias, lost a number of horses in an epizootic of what is believed to have been the same disease as that described in this paper.

History of the Case

The squadron to which this animal belonged had never been stationed at Banias, but when at Metulla 26 days previously the animal was on the same troop line as an animal that suddenly developed alarming symptoms of what is believed to have been the same condition. This latter animal was immediately destroyed. At the time of appearance of clinical symptoms, the animal that is the subject of this paper was at Tel-el-Qadi, some four kilometres from Banias.

The affected horse was one of the best in the squadron, fit and hard, and never missed a patrol. On the day before evidence of the disease was first noted, the animal was out on patrol and showed no sign of any abnormality. On the following morning it was down and unable to rise. The temperature was 104.0° F., and both forelegs appeared to be paralysed. At 4 p.m. on the same day the temperature was 103.0° F. During that day the bladder was emptied by catheterisation, a soap-and-water enema was given, and 4 oz. of sulphanilamide were administered by stomach-tube. On the following morning there was no improvement. The temperature was still 103.0° F., and the animal was transferred by ambulance to Tel-Aviv.

On arrival at Tel-Aviv both forelegs were completely paralysed, conjunctivitis was noted, and slight ulceration of the lower lip. The temperature was 102.6° F. The animal was unable to drink, but took some food. Fæces were passed, but urine was withheld. The condition of the animal gradually deteriorated until 48 hours later, when, unable to swallow and completely helpless, it was destroyed. In all, some 72 hours elapsed between onset of illness and death by destruction.

Laboratory Examination

The only abnormalities noted at post-mortem examination were a subcutaneous gelatinous infiltration of the right flank and congestion of the right lung, both probably due to the animal having lain on that side, and hyperæmia of small vessels in the substance of the brain.

Smears and cultures from spleen, liver and kidney were negative.

Histological examination of cerebral cortex, cerebellum, basal ganglia, mid-brain, pons, medulla, and spinal cord revealed a non-suppurative encephalomyelitis characterised by perivascular accumulations of large and small round cells, foci of reactionary cells in both grey and white matter, marked degenerative changes in nerve cells in all regions, vascular dilation, numerous small hæmorrhages, and congestion of meningeal vessels. The inflammatory reaction appeared to be more intense in the medulla and all regions of the spinal cord than elsewhere. There was no evidence of meningitis.

Degenerative changes were noted in liver cells, especially in the region of central veins, and numerous small hæmorrhages were found in the kidney.

Efforts to transmit the disease to two rabbits, three guinea-pigs and twelve mice by intracranial inoculation of the supernatant fluid after standing overnight of an unfiltered, bacteriologically sterile, 1 : 10 saline emulsion of frontal cortex preserved in 50 per cent. glycerine-saline in the refrigerator for eleven days, were unsuccessful.

Discussion

Histological findings in this case of equine encephalomyelitis bear a striking resemblance to those described by Shirlaw (1940) in cases of the disease in India, and it would appear likely that the disease in India is of the same nature as that now known to exist in Palestine and Syria.

Acknowledgement

I am greatly indebted to Major A. G. Heveningham, Senior Veterinary Officer, Transjordan Frontier Force, for information concerning the disease in Syria, and for details of the history of this case prior to transfer of the sick animal to the Government Veterinary Laboratory, Tel-Aviv.

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ABSTRACT FROM CURRENT LITERATURE

RINDERPEST

THERE has recently been published (*American Journal of Veterinary Research,* 1946, *VII,* No. 23) an account of some valuable work on rinderpest, carried out by a group of American and Canadian workers. The whole of the above issue is devoted to the subject : it contains 16 papers. In order that our readers may be acquainted with this work we reproduce extracts from the introduction and foreword and the titles and summaries of the various papers. To research workers, especially those dealing with viruses and virus infections, we specially recommend a careful perusal of these articles : they are of much interest and value, not only as concerns rinderpest and its control, but also virus work in general.

" The purpose of the project was to develop ways and means of protecting the livestock industry of the North American continent against a foreign plague that might be introduced intentionally, as an enemy method of ' biological warfare ' or accident " ; and for this work six officers of the U.S. Army Veterinary Corps, one from the Medical Corps of the U.S. Navy and two Canadian scientists, together with a corps of technicians from both countries, were employed. There was set up a Joint United States-Canadian Commission, appointed by the United States Secretary of War and the Canadian Minister of National Defence, composed of scientists from both countries.

The work was carried out on an island in the St. Lawrence River on which

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existing buildings were reconstructed "to permit of rigid quarantine and enforcement of safety procedures." Two problems were given to the scientific staff: (1) "To prepare a tissue vaccine according to methods previously developed in order to provide rapidly the means of surrounding an epizootic should it occur with a ring of immunised animals"; (2) "to investigate the possibility of developing an efficient vaccine which could be produced more economically and without requiring the use of large numbers of animals."

The results of all this work are published in the 16 papers. The outstanding feature is the evolving of the "avianised vaccine." It is reported that it is now being tested in a country where the disease is enzootic and we are promised a report when the work is completed. The following quotation gives the position when the journal was published: "It may be stated, however, that the avianised virus has proved to be so attenuated that only mild and negligible reactions are produced in native cattle which are more highly susceptible to the disease than English breeds. At the same time, both native and European breeds are well protected by the vaccine against the virus strain from which the avianised virus was developed and against enzootic virus strains."

The following are the papers and their summaries:—

1. *The Cultivation of Rinderpest Virus in the Developing Hen's Egg.*—Shope, R. E., Griffiths, H. J., and Jenkins, D. L., pp. 135-141.

SUMMARY

(1) Rinderpest virus has been cultivated for 11 serial passages on the chorio-allantoic membranes of embryonating hens' eggs.

(2) The titre of virus grown in eggs compares favourably with the titre of that in bovine tissues ordinarily used in the preparation of anti-rinderpest vaccines.

(3) In eggs inoculated when embryonated for ten days, the virus after three days' growth is present in the membranes but not in the fluids or embryos.

In a single experiment, eight-day eggs that had been incubated for five days after inoculation contained virus, not only in the membranes, but in the embryos as well. There is thus every likelihood that rinderpest virus can eventually be adapted to regular multiplication in the embryos, thereby increasing the potential quantitative yield of virus per egg.

(4) The most favourable incubation temperature for the cultivation of rinderpest virus in eggs seems to be in the neighbourhood of 37° C., 39° C. At 35° C. there is evidence that egg-cultivated virus becomes attenuated, while at 32° C. the virus cannot be maintained in serial egg passages.

2. *Certain Immunity Reactions.*—Walker, R. V. L., Baker, J. A., and Jenkins, D.L., pp. 142-144.

SUMMARY

Serums from calves recovered from rinderpest have the power of fixing complement when mixed with suspensions of spleen containing the virus. However, the titres of such serums are too low to yield dependable results. Rinderpest virus can be neutralised *in vitro* by serums of calves recovered

from rinderpest. Such serums in 1 c.c. amounts will neutralise at least 1,000 minimal infective doses. On the basis of the neutralisation test, a method of diagnosis is discussed.

3. *Immunisation Experiments with Inactivated Bovine Tissue Vaccines.*—Walker, R. V. L., Griffiths, H. J., Shope, R. E., Maurer, F. D., and Jenkins, D. L., pp. 145-151.

SUMMARY

(1) The method of preparation of a satisfactory bovine tissue rinderpest vaccine has been outlined.

(2) Using high titre virus from a single animal, a vaccine of quite good immunising potency was prepared.

(3) Chloroform appeared to be slightly superior to formalin as an inactivating agent for use in vaccine production.

(4) Pooled infective tissues from several animals, prepared as a vaccine "lot," yielded a slightly less potent immunising agent than did high virus titre vaccine from a single animal, probably due to the fact that the initial virus titres of the pooled tissues were lower.

(5) Chloroform-inactivated vaccine remains potent for at least fifteen weeks when stored at refrigerator temperature (± 2° C.), though it loses its immunising capacity after only eight days' incubation at 38° C.

(6) It is recommended that the dose of rinderpest vaccine for use under field conditions be 20 c.c., administered subcutaneously.

4. *Infection of the Embryos and the Fluids of Developing Hen's Eggs.*—Shope, R. E., Maurer, F. D., Jenkins, D.L., Griffiths, H. J., and Baker, J. A., pp. 152-163.

SUMMARY

(1) Rinderpest virus introduced into either the allantoic or amniotic sacs of ten-day embryonated eggs failed to infect the embryos.

(2) Rinderpest virus of direct bovine origin, injected into seven-day embryonated eggs by way of the yolk sac, infected both the embryos and the extra-embryonic fluids. The virus thus established could not be transmitted indefinitely in series in eggs, however, by yolk sac inoculation.

(3) Rinderpest virus that had been subjected to a preliminary series of chorio-allantoic membrane passages was capable of being transmitted indefinitely in series in eggs when administered by way of the yolk sac. In such eggs the virus was widely distributed, being present in the embryos, the extra-embryonic fluids, and the egg membranes. Between 8 and 12 serial chorio-allantoic membrane passages were required, in one experiment in which the question was studied, to adapt the rinderpest virus to yolk sac propagation.

(4) A second adaptive phase through which the rinderpest virus passed upon serial transfer in eggs by yolk sac inoculation was characterised by a shortening of the time elapsing between introduction of the virus into the yolk sac and its eventual appearance in the embryos and extra-embryonic

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fluids. In early serial passages of virus by this route, the virus did not reach the embryo, after being placed in the yolk sac, until between forty-eight and seventy-two hours after inoculation. In later passages, after the second adaptive phase was complete, the virus reached the embryos and extra-embryonic fluids within twenty-four hours of the time it was introduced into the yolk sac.

(5) Rinderpest virus appears to have no constant and dependable lethal effect upon chicken embryos, although many infected embryos do succumb.

(6) Eggs that have been embryonated for six or seven days at time of inoculation are most favourable for use in the propagation of virus by way of the yolk sac. Five-day embryonated eggs are completely unsatisfactory for use and eight- or nine-day embryonated ones are less satisfactory than those of seven days.

(7) The minimal infective dose of rinderpest virus for eggs, either by chorio-allantoic membrane or yolk sac inoculation, is roughly in agreement with the subcutaneous m.i.d. of virus for calves.

(8) Immunologic evidence indicating the identity of the egg-cultivated virus with rinderpest virus of direct bovine origin is presented.

5. *Attempts to Prepare an Effective Rinderpest Vaccine from Inactivated Egg-Cultivated Virus.*—Maurer, F. D., Walker, R. V. L., Shope, E., Griffiths, H. J., Dubois, L., Jenkins, V. C., pp. 164-169.

SUMMARY

(1) Chloroform or formalin-inactivated vaccines prepared from rinderpest virus present in the membranes, yolk sacs, embryos or fluids of infected embryonating eggs failed to induce a satisfactory immunity in calves against rinderpest.

(2) Mixture of a suspension of either infected or normal chicken embryo with known effective bovine tissue vaccine markedly decreased the immunising effectiveness of the bovine vaccine.

(3) Tissues from a calf infected with rinderpest virus that had been subjected to 37 serial passages in eggs, when prepared into chloroformised tissue vaccine, made a preparation that immunised satisfactorily and completely against rinderpest.

(4) It is believed that the evidence presented points to the likelihood that the failure of inactivated egg virus vaccines to immunise against rinderpest is not due to any lack of immunising potency of the virus they contain, but rather to the presence of interfering embryo or egg proteins in the preparations.

6. *The Persistence of Virus in Chicks Hatched from Infected Eggs.*—Shope, R. E., Griffiths, H. J., pp. 170-173.

SUMMARY

(1) Chicks, hatched from eggs infected with rinderpest virus, contain the virus in their brains and in their solid viscera. The virus may persist in such chicks for as long as five days.

(2) We were unsuccessful in infecting baby chicks with rinderpest virus administered by way of the unabsorbed yolk sac, intraperitoneally, intrathoracically, intranasally, subcutaneously or by feeding or scarification.

(3) Rinderpest virus administered intracerebrally to one-day-old chicks is demonstrable in the brains of such birds for at least eight days. It is not transmissible in series directly from chick brain to chick brain.

7. *The Attenuation of Rinderpest Virus for Cattle by Cultivation in Embryonating Eggs.*—Jenkins, V. C., Dubois, L., Shope, R. E., pp. 174-178.

SUMMARY

(1) Prolonged serial passage of rinderpest virus in embryonating eggs attenuates it for calves.

(2) The attenuation appears to be more regular and uniform when the virus is transferred by way of the yolk sac than when it is carried in the chorio-allantoic membrane.

(3) The attenuated virus solidly immunises calves against fully virulent bovine spleen virus. The immunity is effective within ten days.

(4) The mild rinderpest caused by egg-attenuated virus does not appear to be contagious.

(5) Attenuated virus apparently "interferes" with virulent bovine spleen virus, and a calf that received the attenuated agent only four days before the virulent one was protected against the severe and lethal effects of the latter.

(6) If further investigation shows the attenuated virus to be as mild and harmless for calves as preliminary experiments indicate it to be, its use as a "live" vaccine in the field during an emergency is suggested.

(7) "Live" attenuated virus vaccine would give adequate protection during an epizotic more rapidly than would inactivated bovine tissue vaccine.

8. *Rinderpest Infection in Rabbits.*—Baker, J. A., pp. 179-182.

SUMMARY

(1) Rinderpest virus was transferred from calves to rabbits with suspensions of infective spleen given intravenously, but it could not be maintained in a further passage. It was only by alternating the inoculations between calves and rabbits that the virus became pathogenic for rabbits.

(2) This method to increase the pathogenicity of rinderpest virus for a resistant animal would seem applicable to other viruses and an explanation was sought for its success. This is discussed in the text.

(3) Cross-neutralisation tests, using immune serums from calves and rabbits, showed that the illness produced was caused by rinderpest virus and not by another agent encountered in passage.

(4) Contrary to some reports in the literature, and in agreement with others, it is concluded that rinderpest virus can be made to produce definite signs of illness in rabbits.

9. *Neutralisation Tests in Rabbits as a Measure of the Immune Responses in Calves to Vaccination against Rinderpest.* — Jenkins, V. C., Dubois, L., Walker, R. V. L., pp. 183-188.

SUMMARY

A method is outlined of doing neutralisation tests in rabbits that measures

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the antibody content in serums from animals, either vaccinated against or recovered from experimental rinderpest. The practical significance for determining the efficiency of vaccination against rinderpest is discussed.

Of 65 serums tested, 32 were from calves that did not show signs of rinderpest when given a test dose of virus. Of these, 27 possessed neutralising capacity, while five had none. The discussion in the text shows that four of these calves were given poor vaccines that were not expected to produce a good immunity. In only one there was no correlation between immunity and the development of neutralising antibodies. The remaining 33 of 65 serums were taken from uninoculated calves or animals that did not develop immunity after vaccination. None of these serums neutralised the virus. It is concluded that there is a definite correlation between immunity and the development of neutralising antibodies.

10. *The Response of Guinea-pigs to the Virus of Rinderpest.*—Baker, J. A., Terrence, J., Greig, A. S., pp. 189-192.

SUMMARY

Rinderpest virus in three forms—bovine-passaged, lapinised, and avianised—was given intravenously to guinea-pigs. The avianised virus caused no signs of illness and neutralising antibodies were not produced. The lapinised and bovine-passaged types of the virus also caused no signs of illness, but were established and passed serially once as could be demonstrated by inoculations into calves. It was found that the lapinised form was markedly altered in its pathogenic property by one guinea-pig passage. As discussed in the text, these results suggested that the virus caused infection in the usual sense of that word. Furthermore, neutralising antibodies were demonstrated and it was concluded, therefore, that the guinea-pig could be added to the list of laboratory animals susceptible to the virus of rinderpest.

11. *The Survival of Rinderpest Virus in Various Mediums.*—Maurer, F. D., pp. 193-195.

SUMMARY

By determination of the ability of a 1 per cent. suspension of rinderpest virus to survive in various suspending mediums at 36° F., the following facts have been revealed:—

(1) The stability of this virus can vary considerably, depending upon the *pH* and salt concentration of the suspending mediums.

(2) In M/10 phosphate buffer, a suspension of rinderpest virus survived at *pH 7* several days longer than at *pH* 6, and nearly five weeks longer than at *pH* 8.

(3) Under comparable conditions, the virus survived much longer in an M/10 salt concentration than in M/1, M/100, or M/1,000 concentrations.

(4) For maintaining the activity of rinderpest virus in a 10.2 suspension, an M/10 phosphate buffer at *pH 7* was definitely superior to neutral physiologic saline, neutral distilled water, or normal eleven-day egg fluids at *pH* 8.

12. *The Successful Use of Young Chicks to Measure the Concentration of Rinderpest Virus Propagated in Eggs.* — Baker, J. A., Greig, A. S., pp. 196-198.

SUMMARY

Serums were obtained from chicks inoculated intravenously with avianised rinderpest virus that had been lyophilised (vaccine), and then tested for neutralising capacity against lapinised virus by the rabbit method. It was found that neutralising antibodies developed when vaccines containing active virus were given, and when various dilutions were tested the results paralleled those obtained with calves. It was concluded, therefore, that the chick substituted for the calf satisfactorily, when it was desired to determine in either, the presence of concentration of the avianised form of rinderpest virus.

13. *The Production of Rinderpest Vaccine from an Attenuated Strain of Virus.* —Hale, M. W., Walker, R. V. L., pp. 199-211.

SUMMARY

(1) Rinderpest virus, attenuated by culture in embryonated eggs, may be used as a vaccine to immunise cattle against the disease.

(2) The mild symptoms which occasionally result from administration of the vaccine are not transmitted by close and intimate contact.

(3) The attenuated virus does not regain its original bovine pathogenicity under normal conditions, and only experimentally, after a series of six direct blood passages in calves.

(4) A method of producing a stock of attenuated seed virus is described.

(5) Production methods for large quantities of the avianised vaccine are described in detail.

(6) Methods of drying the vaccine to improve the keeping qualities of the product are described.

(7) Bacteriologic and potency standards for the vaccine are briefly mentioned.

14. *Immunisation Experiments with Attenuated Rinderpest Vaccine, including Some Observations on the Keeping Qualities and Potency Tests.*—Hale, M. W., Walker, R. V. L., Maurer, F. D., Baker, J. A., Jenkins, D. L., pp. 212-221.

SUMMARY

An attenuated rinderpest vaccine, produced from cultures of virus in embryonating eggs, has been shown to produce a solid and lasting immunity in cattle. The post-vaccination reaction produced is mild and of short duration. The immune response is prompt, most animals demonstrating neutralising antibodies within ten days. Animals of any age and in any period of pregnancy may be safely vaccinated, but no immunity is conferred to the calves from cows vaccinated during the pregnancy. The dried vaccine, packed in vacuum, will maintain its potency for as long as fifteen months when stored at a temperature of 2° to 5° C. The dried vaccine will not remain potent for long periods at high atmospheric temperatures. It rapidly loses its ability to immunise after being reconstituted from the dry state and should be administered within twelve hours. The

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only completely satisfactory method of determining the potency of the dried vaccine is by administration of the vaccine to susceptible cattle and challenging these animals with fully virulent material at the end of a fourteen-day period.

15. *Morphological Changes in the Blood of Young Cattle During Rinderpest and after Vaccination with Attenuated Virus Vaccine.* — Robey, T. O., Hale, M. W., pp. 222-227.

SUMMARY

Uncomplicated rinderpest in young bovine animals is characterised by a marked leucopenia beginning on the third or fourth day after injection of the virus and persisting for several weeks in those animals which survive. The decrease in white cells appears at approximately the same time that there is a rise in body temperature and prior to the appearance of visible clinical symptoms. There also appears a marked shift to the left in neutrophiles, following the sudden decrease in numbers.

Administration of avianised rinderpest vaccine to young bovine animals resulted in a slight and transitory decrease in white cells on the fifth to the ninth day following injection. This slight decrease in white cells, with prompt return to normal numbers, is believed to be due to the activity of the attenuated virus.

16. *Complement Fixation Test for Rinderpest.*—Cooper, H. K., pp. 228-237.

SUMMARY

A satisfactory complement fixation test for rinderpest is reported. The antigen is easily produced in large quantities and readily standardised. The titre of the antigen is maintained for long periods when stored frozen. Heating the antigen for sixty minutes at 60° C. inactivates the virus, but the antigenicity is unimpaired. The test is of great value in measuring the virus content of eggs used for vaccine production, or seed virus for egg inoculation. Complement-fixing antibodies can be demonstrated regularly in the serum of vaccinated animals, beginning approximately fourteen days after vaccination and persisting for five to six months.

The preservation of complement by freezing and the use of sheep cells preserved with Alserver's solution were found satisfactory.

CORRESPONDENCE

OXFORDSHIRE FEDERATION OF WOMEN'S INSTITUTES

DEAR SIR,

At its Annual Meeting the Oxfordshire Federation of Women's Institutes passed a resolution asking that all cows should be milked out on the sale premises on the morning of sale, to prevent the cruel practice of keeping animals unmilked for long periods so as to give them the appearance of being heavy milkers.

A correspondence has ensued with Breed Societies, the Royal Society for the Prevention of Cruelty to Animals, the Ministry of Agriculture, and others. They are unanimous in "viewing the practice with much concern." They

say that it often does "permanent harm to a cow," they admit that there are great difficulties in "obtaining a prosecution" partly on account of the "practice being engrained in the farming community," and they point out that owing to animals arriving at some sales up to mid-day it would be impossible to enforce a uniform milking of every cow on the sale morning, so that the order could be evaded, and that the cows that had been milked would show to disadvantage.

Only one Breed Society has taken practical steps to discourage this evil by instructing stewards to inspect cows before sale. This is a step in the right direction, but it can only be carried out at Breed Society sales, and not at big public auctions.

Prosecutions have to be made by the Royal Society for the Prevention of Cruelty to Animals under a Bill aimed at cruelty in general, but that makes no mention of "overstocking" of cows, and owing to this and other difficulties it is very hard to obtain a conviction, and the R.S.P.C.A. has to bear all the expenses of the prosecution.

If it is really impossible to legislate effectively to stop this cruel custom, it might be a great help if the Press could inform the public of its prevalence, so that their opinion would be behind the R.S.P.C.A. and so strengthen their hands when making prosecutions.

Yours faithfully,

ELIZABETH BRUNNER, *President.*
H. C. DENEKE, *Chairman.*
O. BROOKE POPHAM, ELSIE C. CORBETT, *Vice-Chairmen.*
SARAH STEVENS, *Hon. Treasurer.*

NEWS

F.A.O. VETERINARY CONFERENCE LONDON, AUGUST, 1946

AT the request of the Food and Agriculture Organisation, a meeting was held in London last week to investigate a problem of great importance to the food supplies of the world.

The widespread ravages of animal plagues in many parts of the world and their effects on food supplies are not generally known to the public in the United Kingdom, where the livestock have for many decades been maintained in comparative freedom from disease. Not only do these pestilences cause enormous loss of livestock products for human consumption, but the casualties in working oxen, buffaloes, horses and so on impede the cultivation of large areas of the most productive agricultural land in many countries. For instance, diseases among buffaloes have led to serious reduction in the rice crop, for mechanical traction cannot be used in the paddy fields. Throughout the world the collection of information on animal epidemics and the co-ordination of methods of controlling them has for some years past been the concern of the International Office of Epizootics in Paris.

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International conferences have been held from time to time in various parts of the world to deal with regional problems, and measures for the control of rinderpest have been advanced by the work of American and Canadian research workers during the war.

The purpose of the meeting was to carry matters a stage further by considering in what way the activities of veterinary organisations all over the world could be properly co-ordinated under the F.A.O. umbrella. Eminent veterinary scientists from America, Australia, France, India, Iran, New Zealand, South Africa and the United Kingdom attended. U.N.R.R.A. and international and imperial veterinary organisations were also represented.

The report of the meeting, which will be submitted to the F.A.O. Conference at Copenhagen in September, recommends that as a further step F.A.O. should set up a Standing Veterinary Committee to advise the Organisation, and to co-operate with the International Office in Paris. It is intended that the Standing Committee should have as wide a scope as possible, and F.A.O. are to be asked to consider the possibility of correlating the existing veterinary bodies under central international machinery in Paris.

NOTE

"The Treatment of Purulent Conjunctivitis in Domesticated Animals with a Mixed Sulphanilamide—Sulphathiazole Powder," a note on the experimental treatment of two cases by Mohamed Fouad Said, D.V.M.S. (Egypt).

Since the paper published in the VETERINARY JOURNAL, August, 1946, Vol. 102, No. 8, pp. 259-262, was submitted for publication on March 24 last, the same method of treatment has been tried on similar affections in human beings.

The results confirm the preliminary conclusions given in the paper.—AUTHOR.

Publishers' Notices

Copy of advertisements should be in the hands of the publishers—Baillière, Tindall and Cox—not later than the 1st of the month, if proof is required.

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